PCT/KR 00/01047
RO/KR

EJU KR00/1047

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출 원 번 호 : 특허출원 1999년 제 48608 호
Application Number

출 원 년 월 일 : 1999년 11월 04일
Date of Application

출 원 인 : 주식회사 엘지화학
Applicant(s)

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

2000 년 06 월 29 일

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【서류명】 특허출원서
【권리구분】 특허
【수신처】 특허청장
【참조번호】 0008
【제출일자】 1999.11.04
【발명의 명칭】 캐스파제 억제제 함유 치료제 조성물
【발명의 영문명칭】 Therapeutic composition comprising caspase inhibitor
【출원인】
【명칭】 주식회사 엘지화학
【출원인코드】 1-1998-001275-0
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【취지】 특허법 제42조의 규정에 의하여 위와 같이 출원합니다. 대리인 최규팔 (인)
【수수료】
【기본출원료】 20 면 29,000 원
【가산출원료】 34 면 34,000 원
【우선권주장료】 0 건 0 원
【심사청구료】 0 항 0 원
【합계】 63,000 원

1019990048608

2000/7/

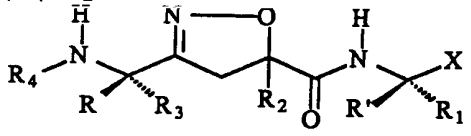
【첨부서류】 1. 요약서·명세서(도면)_1통 2.위임장_1통

【요약서】

【요약】

본 발명은 약제학적으로 허용되는 담체와 함께 유효성분으로서 캐스파제 억제제, 특히 바람직하게는 하기 화학식 1의 이속사졸린 유도체, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체를 함유함을 특징으로 하는 소염 및 세포고사 방지용 치료제 조성물, 및 이 치료제를 제조하는 방법에 관한 것이다:

【화학식 1】



상기식에서

R, R', R_1, R_2, R_3, R_4 및 X 는 명세서에 정의된 바와 같다.

【대표도】

도 1

【명세서】

【발명의 명칭】

캐스파제 억제제 함유 치료제 조성물 {Therapeutic composition comprising caspase inhibitor}

【도면의 간단한 설명】

도 1은 재조합 캐스파제(caspase) 1, 2, 3, 4, 6, 7, 8 및 9에 대한 LB84033의 억제활성을 나타낸 것이고;

도 2는 TNF_{α} 및 악티노마이신(actinomycin) D로 처리하여 세포의 고사가 유도된 랫트 간세포에서 LB84033의 캐스파제 활성 억제효과를 나타낸 것이며;

도 3은 TNF_{α} 및 악티노마이신 D로 처리하여 세포의 고사가 유도된 랫트 간세포에서 LB84033의 세포고사 차단효과를 나타낸 것이고;

도 4는 ConA에 의해 상승된 혈청내 AST 및 ALT 활성에 대한 LB84033의 용량-의존적인 억제활성을 나타낸 것이며(여기에서, 크로스바는 평균치를 나타내며 p값은 student's t-test에 의해 계산한 것이다);

도 5는 ConA에 의해 상승된 세포내 사이토킨류에 대한 LB84033의 용량-의존적인 억제활성을 나타낸 것이고(여기에서, 크로스바는 평균치를 나타내며 p값은 student's t-test에 의해 계산한 것이다);

도 6은 ConA-처리된 마우스 간세포의 형태학적 및 조직학적 변화와 고사성 병변에 대한 LB84033의 억제활성을 나타낸 것이며(여기에서, A 및 G: ConA 및 담체 처리, B 및 H: ConA 및 LB84033 4mg/kg 처리, C 및 I: ConA 및 LB84033 20mg/kg 처리, D 및 J: ConA

및 LB84033 100㎎/㎏ 처리, E 및 K: PBS 및 담체로 처리, F 및 L: PBS 및 LB84033 100㎎/㎏ 으로 처리한 것이고, 각 사진은 그룹당 10마리의 마우스로부터 얻어진 것중 대표적인 것이다);

도 7은 ConA에 의해 유발된 간세포의 고사성 사망으로 인한 PARP 분해에 대한 LB84033의 억제활성을 나타낸 것이고(각 레인은 그룹당 10마리의 마우스에 대해 실험한 결과중 대표적인 것을 나타낸 것이다);

도 8은 감마 인터페론과 항 Fas 항체에 의해 처리되어 고사가 유도된 세포에 대한 본 발명에 따른 화학식 1 화합물의 세포고사 저해효과를 나타낸 것이다.

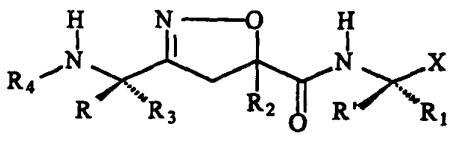
【발명의 상세한 설명】

【발명의 목적】

【발명이 속하는 기술분야 및 그 분야의 종래기술】

<9> 본 발명은 약제학적으로 허용되는 담체와 함께 유효성분으로서 캐스파제 억제제, 특히 바람직하게는 하기 화학식 1의 이속사졸린 유도체, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체를 함유함을 특징으로 하는 소염 및 세포고사 방지용 치료제 조성물, 및 이 치료제 조성물을 제조하는 방법에 관한 것이다:

<10> [화학식 1]

<11> 

<12> 상기식에서

<13> R 및 R' 는 각각 독립적으로 수소, 알킬(-SAC), 사이클로알킬(-SCAC), 방향족

(-Ar), 또는 방향족에 의해 치환된 알킬(-SAC-Ar)을 나타내고;

<14> R_1 은 -SAC, -SCAC, -Ar, -SAC-Ar, 또는 $-CH_2COOH$를 나타내거나, 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내며;

<15> R_3 은 -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(=O)NH_2$ 또는 $-(CH_2)_2C(=O)NH_2$ 를 나타내고;

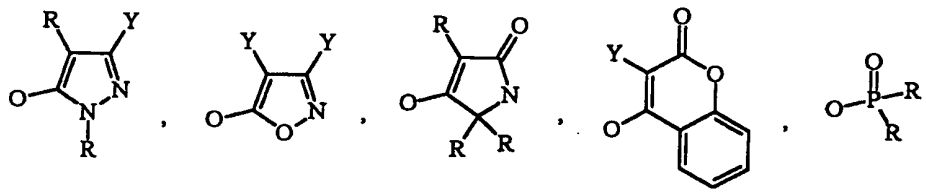
<16> R_2 는 -H, -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-(CH_2)_n(O)_mR_5$ (여기서 R_5 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 0, 1, 2; m = 0, 1), $-(CH_2)_nOC(=O)R_6$ (여기서 R_6 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 1, 2), 또는 $(CH_2)_n(O)_m$ Ar' (여기서 n = 0, 1, 2; m = 0, 1; Ar'=치환된 페닐 또는 이미다졸)을 나타내며;

<17> R_4 는 모든 천연 아미노산의 유기산 아실그룹을 나타내거나, $-C(=O)R_7$ (여기서 R_7 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)OR_8$ (여기서 R_8 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)NR_9R_{10}$ (여기서 R_9, R_{10} = -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-SOR_{11}$ (여기서 R_{11} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), 또는 $-SO_2R_{12}$ (여기서 R_{12} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar)를 나타내고;

<18> R_1 및 인접한 R', R_3 및 인접한 R 은 각각 함께 연결되어 $(CH_2)_n$, $(CH_2)_n$-O-$(CH_2)_m$, 또는 $(CH_2)_n$-NR_{13}-$(CH_2)_m$ [여기서 n+m <9, R_{13} = -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, 또는 -C(=O)-SAC-Ar]의 사이클릭 화합물을 형성할

수 있고;

<19> X 는 -CN, -CHO, $-C(=O)R_{14}$ [여기서 R_{14} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)OR_{15}$ [여기서 R_{15} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-CONR_{16}R_{17}$ [여기서 R_{16} 및 R_{17} 은 각각 -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)CH_2OR_{18}$ [여기서 R_{18} 은 -SAC, -SCAC, -Ar, 또는 -SAC-Ar], 또는 $-C(=O)CH_2OC(=O)R_{19}$ [여기서 R_{19} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar]을 나타내며, X 가 $-COCH_2$-W 인 경우, W 는 $-N_2$, -F, -Cl, -Br, -I, $-NR_{20}R_{21}$ 또는 $-SR_{22}$ [여기서 R_{20}, R_{21} 및 R_{22} 는 각각 독립적으로 -SAC, -SCAC, -Ar, 또는 -SAC-Ar 이거나 R_{20} 및 R_{21} 이 함께 결합하여 사이클릭 화합물을 형성할 수 있다]를 나타내거나; W 는 또한 하기 구조식을 나타낼 수 있고:

<20> 

<21> 상기식에서 Y 는 -OH, OR_{23} (여기서 R_{23} = -SAC, 또는 -SCAC), $-C(=O)R_{24}$ (여기서 R_{24} = -H, -SAC, 또는 -SCAC), -F, -Cl, -Br, -I, -CN, -NC, $-N_3$, $-CO_2H$, CF_3, $-CO_2R_{25}$ (여기서 R_{25} = -SAC, 또는 -SCAC), $-C(=O)NHR_{26}$ (여기서 R_{26} = -SAC, 또는 -SCAC), 또는 $-C(=O)NR_{27}R_{28}$ (여기서 R_{27}, R_{28} = -SAC, 또는 -SCAC) 를 나타낼 수 있고, 차수(order) 및 종류에 무관하게 최대한도로 모노- 또는 폴리-치환될 수 있다.

<22> 상기 화학식 1의 화합물에 대한 설명에서 몇가지 중요한 용어는 다음과 같이 정의된다:

<23> a) 알킬(Simple Alkyl Chain; SAC)은 탄소수 1 내지 8의 탄화수소를 의미하며 측쇄 이성체를 포함한다.

<24> b) 사이클로알킬(Simple CycloAlkyl Chain; SCAC)은 탄소수 3 내지 10의 사이클릭 화합물을 의미한다.

<25> c) 아릴(Ar)은 벤젠[1:2,3,4,5,6], 나프탈렌[1,2:1,2,3,4,5,6,7,8,], 피리딘 [2,3,4:2,3,4,5,6], 인돌[1,2,3,4,5,6,7: 1,2,3,4,5,6,7], 퀴놀린[2,3,4,5,6,7,8: 2,3,4,5,6,7,8], 이소퀴놀린[1,3,4,5,6,7,8: 1,3,4,5,6,7,8], 푸란[2,3:2,3,4,5], 티오펜[2,3:2,3,4,5], 피롤[1,2,3: 1,2,3,4,5], 피리미딘[2,4,5,6: 2,4,5,6], 이미다졸[1,2,4,5:1,2,4,5], 등을 의미하며, 괄호안에서 앞의 수치는 상응하는 아릴 그룹이 본 발명에 따른 억제제에 결합되는 위치를 나타내고, 뒤의 수치는 치환체 Y가 부착되는 위치를 나타낸다

<26> 간세포의 대량 고사(apoptosis)나 간기능의 심각한 손상은 돌발성 간부전증(Fulminant hepatic failure; FHF)과 같은 심각한 임상적 증상을 야기시킨다(참조: Trey, C. et al., 1970, *Progress in liver disease*, Popper, H. and F. Schaffner, eds. Grune and stratton, New York, pp282-298). FHF의 원인으로는 간염바이러스 감염, 약물 및 독소, 알콜, 국소빈혈, 대사성 질환, 악성침윤, 만성 자가면역 간염 등의 여러가지를 들 수 있다. 그러나, 그 메카니즘이 완전히 밝혀지지는 않았다. FHF의 예후는 명확하지 않고 그 진행은 매우 빠르므로 증상이 발현된 후 1-2주일 내에 치명적인 상태에 이르는 일이 빈번하다. 따라서, 치사율은 매우 높다. 그러나, 간병변의 치료는 가역적이며 생존자는 보통 완전히 회복된다.

<27> 지금까지는 FHF를 치료하기 위하여 항생제, 이뇨제, 코르티코스테로이드, 수혈, 약용탄 혈관류 및 혈장분리반출 등의 방법이 사용되었다(참조: Sherlock, S. 1993, *Adv. Intern. Med.* 38: 245-267). 그러나, 이들중의 어떤 방법도 제어된 연구에서 효과적인

것으로 나타나지 않았다. 최근에는 간이식이 상기 증상의 예후를 실제로 개선시킬 수 있는 유일한 치료책으로 인정되었다. 그러나, 면역합병증, 바이러스 또는 세균감염 및 이식편의 입수와 같은 문제로 인하여 간이식 역시 FHF에 대한 완전한 치료방법은 될 수 없다. 따라서, 병이 급진전되고 있는 중에 대량사망으로부터 간세포를 보호할 수 있는 강력한 치료제의 개발이 간절히 요구되는 실정이다.

<28> 고사는 특정의 세포기관에 의해 달성되며, 일련의 서로 명확히 구별되는 형태학적 및 생화학적 변화에 의해 특징지어지는 세포사망의 한 유형이다. 고사는 과도하게 존재하는, 불필요하고 유해한 세포를 제거하며 항상성을 유지하기에 필수적인 과정이면서, 동시에 부적합한 고사는 신경변성질환, 국소빈혈성 손상, 자가면역질환, 여러형태의 암과 같이 많은 인체 질환을 유발시킨다. 바이러스성 간염, 알콜성 간염 및 돌발성 간염에서의 급성 간부전증에서 간세포의 고사가 간손상의 주된 원인이라는 사실은 분명해졌다. 고사신호를 받은 세포내에서는 많은 변화가 일어나며 캐스파제로 불리는 일련의 시스테인 프로테아제에 의해 복잡한 생화학적 반응이 진행된다. 캐스파제는 살아있는 세포가 고사되는 것을 방지하는 단백질, 예를들어 DNA 단편화에 작용하는 뉴클레아제의 억제제인 I^{CAD}/DFF45과 Bcl-2를 불활성화시킨다. 또한, 캐스파제는 세포구조를 직접 해체시킬 뿐아니라 세포골격의 조절에 관여하는 여러 단백질들을 분해하여 간접적으로 세포 구조를 재구성함으로써 고사에 기여한다. 캐스파제는 고사신호를 증폭시키고 전파시키며, 세포 생존에 필요한 세포내 단백질들을 분해시킴으로써 고사 프로그램을 실행시킨다. 이러한 캐스파제의 활성화는 여러 형태의 고사를 시작하게하고, 진행시키고, 종결시키는 과정과 밀접하게 관련되어 있으므로 과도한 고사 또는 불충분한 고사에 기인하는 질환을 치료하기 위한 매력적인 잠재적 표적으로 인식되어 왔다.

<29> 여러종류의 캐스파제 억제제가 개발된 바 있다. 바이러스성 억제제에는 CrmA, p35, IAP 패밀리(고사 억제제) 및 B형 간염 바이러스에 의해 코드화된 HBx 단백질과 같이 4가지의 구별되는 그룹이 있다(참조: Gottlob, K. et al., 1998, *J. Biol. Chem* 273: 33347-33353). 그러나, 이들은 치료제로서 적합하지 못하다. z-VAD-fmk, z-DEVD-fmk 및 Ac-YVAD-cmk와 같은 펩타이드성 캐스파제 억제제는 연구목적으로 폭넓게 이용되고 있으며, 이들 억제제는 세포수준(참조: Sane, A. T. et al., 1998, Cancer Res. 58: 3066-3072), Fas 또는 TNF$_\alpha$에 의해 유발된 간질환의 설치류 모델(참조: Kunstle, G. et al., 1997, *Immunol. Lett.* 55: 5-10), 또는 간이식후의 국소빈혈(참조: Cursio, R. et al., 1999, *FASEB J.* 13: 253-261)에서 고사-차단 활성을 보인다. 페탁(Petak) 등은 관능성(bifunctional) 항암제 BCNU(1,3-비스(2-클로로에틸)-1-니트로소우레아)가 캐스파제 억제활성을 보유하며 시험관내에서 약물-유발성 고사를 억제한다고 보고하였다(참조: Petak, I. et al., 1998, *Cancer Res.* 58: 614-618). 최근에는 사이클로옥시게나제-2(COX-2) 억제제가 FHF의 잠재적인 치료제로서 주목받고 있다(참조: McCormick, P. A. et al., 1999, *Lancet* 353: 40-41). 그러나, 이 물질의 효과에 대해서는 아직 임상적으로 확인되지 않았다.

<30> 한편, 간질환에 대한 신약의 개발은 일차적으로 인체에 있어서의 간염이나 간세포성 손상과 관련된 적합한 동물모델에 의존한다. 따라서, 인간 FHF에 대한 치료제 후보물질의 효과를 검증하기 위해 적합한 동물모델을 채택하는 것은 매우 중요하다. 현재까지 보고된 실험적 간염모델에는 두가지가 있다. 하나는 박테리아 리포폴리사카라이드와 D-갈락토스아민에 의해 유발된 간손상이고(참조: Galanos, C. et al., 1979, *Proc. Natl. Acad. Sci.*, 76: 5939; Lehman, V. et al., 1987, *J. Exp. Med.* 165-657), 다른

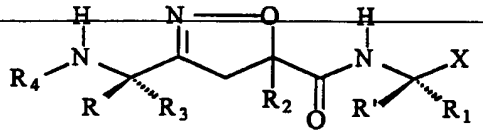
하나는 최근에 개발된 것으로서 ConA-유발성 간염이다(참조: Tiegs, G. et al., 1992, . 90: 196-203; .Mizuhara, H. et al., 1994, *J. Exp. Med.* 179: 1529-1537). ConA는 T 림프구를 활성화시킴으로써 마우스에서 고사성 및 괴사성 세포사망을 일으키는 간질환을 유발시키며, ConA-유발성 간염모델은 많은 점에서, 특히 병인에 있어서의 Fas의 역할 측면에서 인간 FHF와 유사하다. Fas는 간세포에서 많이 발현되며, FasL은 활성화된 T 세포에서 발현되어 세포독성 림프구의 주효인자로서 기능한다. 성숙 마우스에 아고니스트성 모노클로날 항-Fas 항체를 주입하면 급속한 간질환을 야기시키는데, 이는 비정상적으로 활성화된 Fas-FasL 시스템이 세포독성 T 세포와 같은 면역시스템을 활성화시켜 돌발성 인체 간염을 일으킬 수 있음을 나타낸다. FHF의 병인에 특정 CTLs가 관여한다거나, 시험관내에서 Fas-매개된 고사에 대한 일차적 간세포의 민감성, 및 인간 간염바이러스에 의해 형질전환된 간세포에서의 Fas의 과발현과 같은 축적된 데이타가 상기 가설과 일치하고 있다. 최근의 연구에서는 Fas-FasL 시스템의 활성화가 ConA-유발성 간염에 의한 간세포의 손상에 중요한 역할을 하는 것으로 입증되었다(참조: Tagawa, Y. et al., 1998, *Eur. J. Immunol.* 28: 4105-4113). FasL은 ConA가 주입된 직후 간에서 유도되며, 간내부의 T 세포상에서 우세하게 발현되는데, 이는 Fas-FasL 시스템이 ConA-유발성 간염의 진전에 중요한 인자임을 가리킨다. 또한, ConA-간염은 IL-2, IFN$_\gamma$, TNF$_\alpha$, IL-6, IL-4 및 IL-10과 같은 다양한 사이토킨류의 생산과 관련되어 있다.

【발명이 이루고자 하는 기술적 과제】

<31> 이러한 기술적 배경하에 본 발명자들은 세포의 대량고사에 기인하는 돌발성 간부전증과 같은 질환에 대한 효과적인 치료제를 개발하기 위해 집중적인 연구를 수행하였으며, 그 결과 생체내에서 캐스파제 억제제로서 작용하는 물질, 특히 바람직하

게는 하기 화학식 1의 비펩타이드성 이속사졸린 유도체를 이용하는 경우 본 발명의 목적이 완벽하게 달성될 수 있음을 발견하고 본 발명을 완성하게 되었다:

<32> [화학식 1]

<33> 

<34> 상기식에서

<35> R 및 R' 는 각각 독립적으로 수소, 알킬(-SAC), 사이클로알킬(-SCAC), 방향족(-Ar), 또는 방향족에 의해 치환된 알킬(-SAC-Ar)을 나타내고;

<36> R_1 은 -SAC, -SCAC, -Ar, -SAC-Ar, 또는 $-CH_2COOH$를 나타내거나, 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내며;

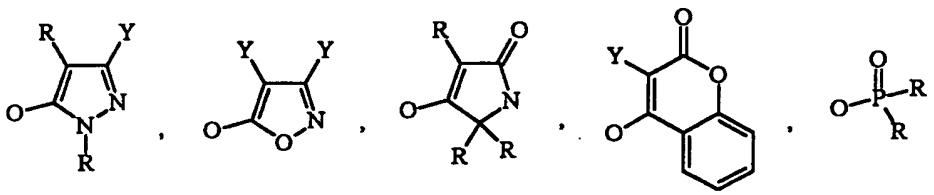
<37> R_3 은 -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(=O)NH_2$ 또는 $-(CH_2)_2C(=O)NH_2$ 를 나타내고;

<38> R_2 는 -H, -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-(CH_2)_n(O)_mR_5$ (여기서 R_5 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 0, 1, 2; m = 0, 1), $-(CH_2)_nOC(=O)R_6$ (여기서 R_6 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 1, 2), 또는 $(CH_2)_n(O)_m$ Ar' (여기서 n = 0, 1, 2; m = 0, 1; Ar'=치환되거나 비치환된 페닐 또는 이미다졸)을 나타내며;

<39> R_4 는 모든 천연 아미노산의 유기산 아실그룹을 나타내거나, $-C(=O)R_7$ (여기서 R_7 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)OR_8$ (여기서 R_8 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)NR_9R_{10}$ (여기서 R_9, R_{10} = -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-SOR_{11}$ (여기서 R_{11} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), 또는 $-SO_2R_{12}$ (여기서 R_{12} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar)를 나타내고;

<40> R_1 및 인접한 R', R_3 및 인접한 R 은 각각 함께 연결되어 $(CH_2)_n$, $(CH_2)_n$-O-$(CH_2)_m$, 또는 $(CH_2)_n$-NR_{13}-$(CH_2)_m$ [여기서 n+m <9, R_{13} = -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, 또는 -C(=O)-SAC-Ar]의 사이클릭 화합물을 형성할 수 있고;

<41> X 는 -CN, -CHO, $-C(=O)R_{14}$ [여기서 R_{14} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)OR_{15}$ [여기서 R_{15} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-CONR_{16}R_{17}$ [여기서 R_{16} 및 R_{17} 은 각각 -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)CH_2OR_{18}$ [여기서 R_{18} 은 -SAC, -SCAC, -Ar, 또는 -SAC-Ar], 또는 $-C(=O)CH_2OC(=O)R_{19}$ [여기서 R_{19} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar]을 나타내며, X 가 $-COCH_2$-W 인 경우, W 는 $-N_2$, -F, -Cl, -Br, -I, $-NR_{20}R_{21}$ 또는 $-SR_{22}$ [여기서 R_{20}, R_{21} 및 R_{22} 는 각각 독립적으로 -SAC, -SCAC, -Ar, 또는 -SAC-Ar 이거나 R_{20} 및 R_{21} 이 함께 결합하여 사이클릭 화합물을 형성할 수 있다]를 나타내거나; W 는 또한 하기 구조식을 나타낼 수 있고:

<42> 

<43> 상기식에서 Y 는 -OH, OR_{23} (여기서 R_{23} = -SAC, 또는 -SCAC), $-C(=O)R_{24}$ (여기서

R_{24} = -H, -SAC, 또는 -SCAC), -F, -Cl, -Br, -I, -CN, -NC, $-N_3$, $-CO_2H$, CF_3, $-CO_2R_{25}$ (여기서 R_{25} = -SAC, 또는 -SCAC), $-C(=O)NHR_{26}$ (여기서 R_{26} = -SAC, 또는 -SCAC), 또는 $-C(=O)NR_{27}R_{28}$ (여기서 R_{27}, R_{28} = -SAC, 또는 -SCAC) 를 나타낼 수 있고, 차수(order) 및 종류에 무관하게 최대한도로 모노- 또는 폴리-치환될 수 있다.

【발명의 구성 및 작용】

<44> 따라서, 본 발명은 약제학적으로 허용되는 담체와 함께 유효성분으로서 캐스파제 억제제를 함유함을 특징으로 하는 소염 및 세포고사 방지용 치료제 조성물을 제공함을 목적으로 한다.

<45> 보다 구체적으로, 본 발명은 상기와 같은 소염 또는 세포고사의 방지 효과에 의거하여 치매, 뇌졸중, AIDS로 인한 뇌손상, 당뇨, 위궤양, 간염바이러스에 의한 뇌손상, 인간 돌발성 간부전증(Fulminant hepatic failure; FHF), 패혈증(septic shock), 장기이식거부반응, 류마티스성 관절염, 허혈성 심장질환에 의한 심장세포괴사의 치료 효과를 갖는 치료제 조성물을 제공한다.

<46> 특히, 본 발명에 따른 조성물은 인간 돌발성 간부전증의 치료제로서 바람직하게 사용될 수 있다.

<47> 본 발명은 또한, 캐스파제 억제제를 사용함을 특징으로 하여 소염 및 세포고사 방지용 치료제 조성물을 제조하는 방법을 제공한다.

<48> 본 발명의 바람직한 태양은 유효성분인 캐스파제 억제제로서 상기 화학식 1의 이속사졸린 유도체, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체를 사용하는 치료제 조성물이다.

<49> 상기 화학식 1의 이속사졸린 유도체는 경우에 따라 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체 형태로 사용될 수 있다.

<50> 화학식 1의 화합물 중에서도 본 발명에 따른 용도에 특히 적합한 화합물은 R 및 R'이 수소를 나타내고; R_1 이 $-CH_2COOH$ 또는 $-CH_2COOCH_3$ 를 나타내며; R_2 가 수소, 메틸 또는 $(CH_2)_n(O)_mAr'$[n=1, 2; m=0, 1; Ar' = 치환되거나 비치환된 페닐 또는 이미다졸]을 나타내고; R_3 가 $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(O)NH_2$ 또는 $-(CH_2)_2C(O)NH_2$ 를 나타내며; R_4 가 $-C(=O)(O)_nR_{29}$[n=0, 1; R_{29} = -Ar 또는 -SAC-Ar], $-SO_2R_{30}$[R_{30} = -Ar 또는 -SAC-Ar], 또는 $-C(=O)NHR_{31}$[R_{31} = -Ar 또는 -SAC-Ar]을 나타내고; X 는 $-C(=O)CH_2N_2$, $-C(=O)CH_2Br$, $-C(=O)CH_2Cl$, $-C(=O)CH_2OAr'$[Ar' = 페닐] 또는 $-C(=O)CH_2OC(=O)Ar''$[Ar'' = 2,6-디클로로페닐 또는 2,6-디메틸페닐]을 나타내는 화합물이다.

<51> 보다 특히 적합한 화합물은

<52> (3S)-3-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-펜타노산;

<53> (3S)-3-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-5-페녹시메틸-4,5-디하이드로-이속사졸-5-카보닐-아미노}-4-케토-펜타노산;

<54> (2S)-2-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-5-페녹시메틸-4,5-디하이드로-이속사졸-5-카보닐-아미노}-숙신산 1-(N-메틸-N-메톡시)-아미드;

<55> (3S)-3-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<56> (3S)-3-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-4,5-디하이드로-이속

사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<57> (3S)-3-{3-[(1S)-1-페닐메틸옥시카보닐아미노-2-메틸-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

~~<58> (3S)-3-{3-[(1S)-1-(나프탈렌-1-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;~~

<59> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<60> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<61> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<62> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<63> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<64> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<65> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<66> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디

하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<67> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

~~<68> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페닐-메틸-4,5-디~~하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<69> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-3-카복시-프로필]-5-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<70> (3S)-3-{3-[(1S)-1-(퀴놀린-2-일-카보닐아미노)-2-메틸-프로필]-5-페녹시메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-펜타노산;

<71> (3S)-3-{3-[(1S)-1-(나프탈렌-2-설포닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<72> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2-나프틸옥시)-펜타노산;

<73> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-5-페녹시-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(1-나프틸옥시)-펜타노산;

<74> (3S)-3-{3-[(1S)-1-(2S)-2-아세틸아미노-숙시노일아미노)-3-카복시-프로필]-5-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<75> (3S)-3-{3-[(1S)-1-(나프탈렌-2-카보닐아미노)-2-메틸-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2-나프틸옥시)-펜타노산;

<76> (3S)-3-{3-[2-메틸-(1S)-1-(2-나프탈렌카보닐아미노)-프로필]-4,5-디하이드로-이속

사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산 (부분입체이성체 혼합물);

<77> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸옥시카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<78> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸옥시카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<79> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸옥시카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<80> (3S)-3-{3-[2-메틸-(1S)-1-(페닐에틸카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<81> (3S)-3-{3-[2-메틸-(1S)-1-(페닐에틸카보닐아미노)-프로필]-5-페닐메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<82> (3S)-3-{3-[2-메틸-(1S)-1-(페닐에틸카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<83> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<84> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<85> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<86> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌설포닐아미노)-프로필]-5-페닐-메틸-4,5-디

하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산 (부분입체이성체 혼합물);

<87> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌설포닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산 (부분입체이성체 혼합물);

<88> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈렌설포닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산 (부분입체이성체 혼합물);

<89> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)에틸카보닐아미노)-프로필]-5-페닐- 메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<90> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)에틸카보닐아미노)-프로필]-5-페닐- 메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<91> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)에틸카보닐아미노)-프로필]-5-페닐- 메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<92> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)메틸카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<93> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)메틸카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<94> (3S)-3-{3-[2-메틸-(1S)-1-((3-인돌릴)메틸카보닐아미노)-프로필]-5-페닐-메틸

-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<95> (3S)-3-{3-[2-메틸-(1S)-1-(신나모일아미노)-프로필]-5-페닐메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<96> (3S)-3-{3-[2-메틸-(1S)-1-(신나모일아미노)-프로필]-5-페닐메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<97> (3S)-3-{3-[2-메틸-(1S)-1-(신나모일아미노)-프로필]-5-페닐메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<98> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸설포닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<99> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸설포닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<100> (3S)-3-{3-[2-메틸-(1S)-1-(페닐메틸설포닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<101> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<102> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<103> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<104> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<105> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-디아조-펜타노산;

<106> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-브로모-펜타노산;

<107> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(2,6-디클로로벤조일옥시)-펜타노산;

<108> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<109> (3S)-3-{3-[2-메틸-(1S)-1-(퀴놀린-2-일-카보닐아미노)-프로필]-5-(1-이미다졸릴-메틸)-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<110> (3S)-3-{3-[2-메틸-(1S)-1-(2-나프탈렌카보닐아미노)-프로필]-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-펜타노산;

<111> (3S)-3-{3-[(1S)-1-(숙시노일아미노)-3-카복시-프로필]-5-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시-펜타노산;

<112> (3S)-3-{3-[2-메틸-(1S)-1-(숙시노일아미노)-프로필]-5-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-페녹시펜타노산; 또는

<113> (3S)-3-{3-[2-메틸-(1S)-1-(1-나프탈레닐카보닐아미노)-프로필]-5-페닐-메틸-4,5-디하이드로-이속사졸-5-카보닐아미노}-4-케토-5-(1-피페리디닐)-펜타노산이다.

<114> 화학식 1의 비펩타이드성 이속사졸린 유도체는 본 발명자들에 의해 개발이 완료된 것으로서, 그 화합물의 제조방법은 대한민국 특허출원 제98-11787호 및 이 출원을 우선권 주장하여 국제출원된 PCT/KR99/00561호에 구체적으로 기재되어 있다.

<115> 본 발명자들은, 하기 설명하는 바와 같이, 화학식 1 화합물의 시험관내 및 생체내 캐스파제 억제활성을 측정하였으며, ConA 또는 TNF$_\alpha$/악티노마이신 D에 의해 간질환이 유발된 경우 간세포의 세포생존율, 간염의 치료효과, 간세포 고사의 감소 및 PARP 분해의 억제 등에 대한 화학식 1 화합물의 활성을 조사하였다. 본 발명자들은 또한, 감마 인터페론 및 항 Fas 항체에 의해 세포고사가 유도된 경우 세포의 생존율에 대한 화학식 1 화합물의 효과를 공지의 캐스파제 억제제 Ac-DEVD-CHO 또는 z-DEVD-cmk와 비교하여 실험하였다. 좀더 구체적으로 설명하면 다음과 같다.

<116> 먼저, 마우스에서 ConA에 의해 유발된 급성 간질환에 대한 화학식 1의 화합물의 억제활성을 조사하였다. 그 결과, 화학식 1의 비펩타이드성 억제제는 캐스파제 억제활성 뿐아니라 시험관내 및 생체내 간세포의 고사성 사망에 대한 억제활성을 나타내었는데, 이는 간세포의 대량고사에 의해 유발된 인간 FHF에 대해 화학식 1의 화합물이 치료제로서 사용될 수 있음을 시사한다.

<117> 광범위한 캐스파제 억제활성을 보유하고 있는 화학식 1의 화합물은 애초에 캐스파제 패밀리 효소의 특이적인 억제제로서 고안되었다는 점에서 BCNU(1,3-비스 (2-클로로에틸)-1-니트로소우레아) 또는 COX-2(사이클로옥시게나아제-2)와는 다르다. 고사과정은 매우 복잡하게 진행되며, 이 과정의 여러단계에 캐스파제가 관여한다. 또한, 많은 공지의 기질들이 우연히 발견되었기 때문에 캐스파제의 조절에 대해서는 알려진 것이 거의 없다. 따라서, 급성 FHF 중에 간세포의 대량고사를 단기간에 차단하기 위해서는 특

이적 캐스파제 억제제에 비해 광범위한 캐스파제 억제활성을 갖는 물질이 보다 효과적으로 작용할 수 있다. 이점에서 화학식 1의 화합물은 치료제 후보물질로서 이상적이다.

<118> 본 발명자들은 캐스파제 억제제인 화학식 1의 화합물의 고사-차단효과를 확인하기 에 적합한 실험모델로서 ConA-유발성 간염모델을 이용하였다. ConA-유발성 간염에는 여러 사이토킨류, 예를들어 IL-2, IFN$_\nu$, TNF$_\alpha$, IL-6, IL-4 및 IL-10이 관여한다. 본 발명에서는 ConA에 의해 증가된 혈청중의 IL-1$_\beta$, IL-2, IL-4 및 IFN$_\nu$의 농도에 대한 화학식 1의 화합물의 효과를 테스트하였으며, 그 결과 이 화합물이 도 1 및 2에 나타낸 바와 같은 그의 캐스파제-1-억제활성으로 인하여 용량-의존적인 방식으로 IL-1$_\beta$수준을 상당히 억제하는 것으로 확인되었다(도 5A 참조). 그러나, 화학식 1의 화합물은 IL-2, IL-4 및 IFN$_\nu$에 대해서는 별다른 영향을 미치지 못하였는데(도 5B, C, D 참조), 이는 화학식 1의 화합물이 캐스파제 억제제로서 활성을 발휘하는 주요 대상이 Fas를 발현하는 간세포라는 사실에 기인한다. 즉, 화학식 1의 화합물은 캐스파제와 관련된 고사로부터 간세포를 보호하지만, 활성화된 T 세포를 직접적으로 억제하지는 못한다.

<119> 세포에서 캐스파제-3-유사 프로테아제에 대한 생기질(biosubstrate)중의 하나는 PARP(116kDa)이며, 이것은 고사중의 세포에서 85kDa 및 31kDa 단편으로 분해된다. 따라서, PARP의 85kDa 분해물은 고사의 초기지표로서 이용되어 왔다 (Lazebnik, Y. A. et al., 1994, *Nature* 371: 346-347; Kaufmann, S. H. et al., 1993, *Cancer Res.* 53: 3976-3985). 화학식 1의 화합물은 ConA에 의해 유발된 간세포의 고사로 인한 PAPR의 분해를 용량-의존적인 방식으로 억제하였다(도 7 참조). 웨스턴 블롯 분석에서, 85kDa 분해산물의 양은 화학식 1의 화합물 투입량이 증가함에 따라 점차 감소하였고, 온전한 116kDa PARP는 상대적으로 일정한 것으로 나타났다.

<120> 도 6으로부터 알 수 있듯이, ConA는 간세포에 심각한 형태학적 및 조직학적 변화를 일으키며, 고사성 병변도 명확히 관찰된다. 그러나, 간세포의 대부분은 여전히 생존하며 고사세포는 일부에 불과하다. 이러한 현상에 의해 온전한 116kDa PARP가 ConA/비히클 마우스의 간에서도 나타나는 이유를 이해할 수 있다.

<121> 한편, 본 발명자들은 감마 인터페론 및 항 Fas 항체로 처리함으로써 인간유래의 세포주(Fas responsive cell)에 세포고사를 유도한 후, 이 세포에 대한 화학식 1의 화합물의 세포고사 저해효과를 확인하기 위한 실험을 수행하였으며, 그 결과, 화학식 1의 화합물이 공지의 캐스파제-3 저해제인 Ac-DEVD-CHO 또는 z-DEVD-cmk에 비해서도 2배 이상 우수한 세포고사 저해효과를 나타냄을 확인할 수 있었다(동일농도에서의 세포생존율이 Ac-DEVD-CHO : 35.1%, z-DEVD-cmk : 47.3%, LB84033 : 100%, 표 1 및 도 8 참조).

<122> 이상의 실험결과를 통해, 화학식 1의 비펩타이드성 화합물은 폭넓은 캐스파제 억제활성을 지니고 있고, 이에 따라 소염 및 세포고사의 차단효과를 지니고 있으며, 특히 인간 FHF에서 간세포의 대량고사를 차단하는 치료제로서 효과적으로 사용될 수 있음을 알 수 있다.

<123> 캐스파제 억제제, 특히 화학식 1의 화합물은 목적하는 바에 따라 다양한 약제학적 투여형태로 제형화될 수 있다. 본 발명에 따른 약제학적 조성물을 제조함에 있어서는, 유효량의 캐스파제 억제제, 특히 화학식 1의 화합물 또는 그의 염을 제조하고자 하는 제형에 따라 선택될 수 있는 다양한 약제학적으로 허용되는 담체와 혼합한다.

<124> 캐스파제 억제제 물질은 목적하는 바에 따라 주사용 제제, 경피용 제제 또는 경구용 제제로 제형화될 수 있으며, 투여의 용이성 및 용량의 균일성 측면에서 바람직하게는 단일투여형으로 제조된다.

<125> 경구용 제제를 제조하는 경우에는 통상의 약제학적 담체를 사용할 수 있다. 예를들어, 현탁액, 시럽제, 엘릭시르 및 용액제와 같은 경구용 액체 제제의 경우 물, 글리콜, 오일, 알콜 등을 담체로 사용할 수 있고; 산제, 환제, 캅셀제 및 정제와 같은 고체 제제의 경우에는 전분, 설탕, 카올린, 윤활제, 결합제, 붕해제 등을 사용할 수 있다. 투여의 용이성으로 인하여 정제 및 캅셀제가 가장 편리한 복용형태이며, 정제 및 환제는 장피제로 제조하는 것이 바람직하다.

<126> 비경구 제제의 경우 담체로는 통상 멸균수를 사용하며, 용해보조제와 같은 다른 성분도 포함시킬 수 있다. 주사용 제제, 예를들면 멸균 주사용 수성 또는 유성 현탁액은 공지된 기술에 따라 적합한 분산제, 습윤제, 또는 현탁제를 사용하여 제조할 수 있다. 이를 위해 사용될 수 있는 용매에는 물, 링거액 및 등장성 NaCl 용액이 있으며, 멸균 고정오일도 통상적으로 용매 또는 현탁 매질로서 사용한다. 모노-, 디-글리세라이드를 포함하여 어떠한 무자극성 고정오일도 이러한 목적으로 사용될 수 있으며, 또한 올레산과 같은 지방산도 주사용 제제에 사용할 수 있다.

<127> 경피 제제의 경우에는 담체로서 침투촉진제 및/또는 적당한 습윤제를 임의로 피부에 대한 자극성이 없는 적당한 첨가제와 함께 사용할 수 있다. 첨가제로는 피부를 통한 투여를 촉진시키고/시키거나 목적하는 조성물을 제조하는데 도움이 되는 것을 선택한다. 경피 제제는 경피용 패취, 점적제 또는 연고와 같은 다양한 방식으로 투여된다.

<128> 캐스파제 억제제, 특히 화학식 1의 화합물을 임상적인 목적으로 투여시에 단일용량 또는 분리용량으로 숙주에게 투여될 총 일일용량은 체중 1kg 당 0.1 내지 100㎎의 범위가 바람직하나, 특정 환자에 대한 특이 용량 수준은 사용될 특정 화합물, 환자의 체중, 성, 건강상태, 식이, 약제의 투여시간, 투여방법, 배설률, 약제혼합 및 질환의 중증도

등에 따라 변화될 수 있다.

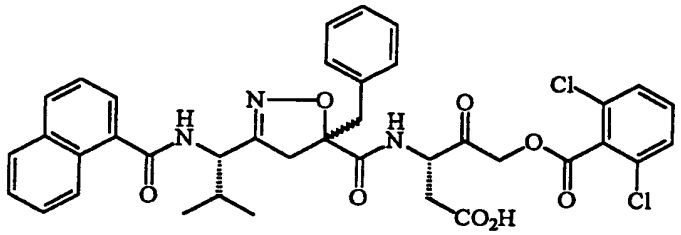
<129> 이하, 본 발명의 효과를 하기 실시예를 통하여 보다 구체적으로 설명한다. 그러나, 이들 실시예는 본 발명에 대한 이해를 돕기위한 것일 뿐, 어떤 의미로도 본 발명의 범위를 제한하는 것은 아니다.

【발명의 효과】

<130> 하기 실시예에서 사용된 ConA는 베링거 만하임 게엠베하(Germany)에서 구입하였고; 캐스파제의 펩타이드계 기질 Ac-YVAD-pNA(캐스파제-1), Ac-VDVAD-pNA (캐스파제-2), Ac-DEVD-pNA(캐스파제-3, 7, 8 및 9), Ac-LEVD-pNA(캐스파제-4) 및 Ac-VEID-pNA(캐스파제-6), 그리고 펩타이드계 캐스파제 억제제 Ac-DEVD-fmk 및 z-VAD-CHO 는 알렉시스 컴파니(Alexis Co., San Diego, CA)에서 구입하였으며; Ac-DEVD-CHO 및 z-DEVD-cmk는 Bachem 사에서 구입하였고; 항 Fas 항체는 온코르(Oncor)사 (Cat#A8050)에서 구입하였으며; WI38 세포는 ATCC에서 구입하였고; IFN-gamma는 LG Pharmaceuticals에서 구입하였으며; 그밖에 모든 세포 배양 매질 및 첨가물들은 Gibco BRL(Tsuen Wan, Hong Kong)에서 구입하였다. 재조합 인간 캐스파제는 논문(Garcia-Calvo M et al., 'Purification and catalytic properties of human caspase family members', *Cell Death Differ,* 1999, Apr; 6(4): 362-9)에 기재된 방법을 참고하여 제조할 수 있으며, 특히 캐스파제-3, 6, 7 및 8은 시판되고 있다(Pharmingen, SanDiego, CA, USA, 캐스파제-3: 66281T, 캐스파제-6: 66291T, 캐스파제-7: 66301T, 캐스파제-8: 66311T).

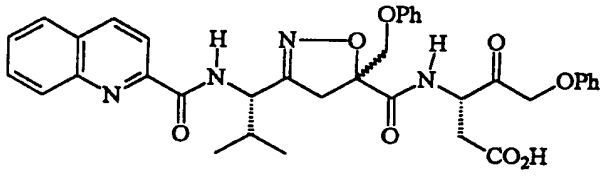
<131> 하기 실시예에서 LB84033, LB84022 및 LB84028은 각각 화학식 1의 화합물에 속하는 하기 구조식의 화합물을 나타낸다.

<132>



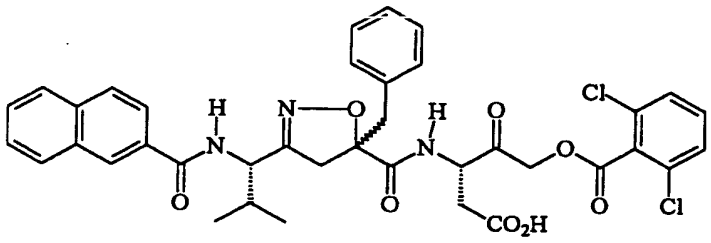
(LB84033)

<133>



(LB84022)

<134>



(LB84028)

<135> 실시예 1: 간세포의 분리 및 배양

<136> 수컷 스프라그-도울리(Hanlan Sprague-Dawley) 랫트를 문헌(참조: MacMicking et al., 1995, *Cell* 81: 641-650)에 기재된 방법에 따라 사육하였다. 랫트의 간세포를 문헌(Stadler et al., *Arch. Biochem. Biophys.* 302: 4-11) 기재의 방법에 따라 분리, 정제 및 배양하였다. 고도로 정제된 간세포(순도 98% 이상, 트리판 블루(trypan blue) 제거법에 의한 생존율 98% 이상)를 10% 소혈청을 함유하며 HEPES 15mM(pH 7.4), 인슐린 1㎛M , L-글루타민 2mM, 페니실린 100단위/㎖ 및 스트렙토마이신 100㎍/㎖가 보충된 윌리암스 배지 E(Williams medium E)에 현탁시켰다. 세포 생존율 시험을 수행하기 위해서는 콜라겐에 의해 코팅된 12-웰 플레이트에 세포를 웰당 2×10^5세포 밀도로 가하였고, 효소활성 측정을 위해서는 100㎖ 디쉬당 5×10^6세포의 밀도로 세포를 가하였다.

<137> 실시예 2: 무세포 시스템에서 LB84033의 캐스파제 억제활성(시험관내 실험)

<138> 테트라펩타이드계 발색성 기질에 대한 각 효소의 가수분해 정도를 측정함으로써 재조합 캐스파제 활성을 분석하였다. 재조합 인간 캐스파제를 에세이 완충액 (글리세롤 20%(v/v)를 함유하는 HEPES 100mM, pH 7.4)중에서 DTT 5mM과 함께 실온에서 20분간 예비배양하였다. 효소분획(405nm/h에서의 흡광도가 0.2에 해당하는 효소활성을 함유, 2㎕)을 96-웰 플레이트에서 LB84033 100㎛M의 존재 또는 부재하에 200㎛M의 발색성 기질 150㎕와 혼합하였다. 이 혼합물을 37℃에서 방치하였다. 마이크로플레이트 리더(reader)에서 1시간동안 효소적으로 유리된 pNA의 흡광도를 405nm에서 불연속적으로 측정하였다. 초기속도(n=3)로부터 캐스파제 활성을 계산하였다(평균치±SE)(n=4, 여기서 n은 실험의 반복횟수를 나타낸다).

<139> 그 결과, LB84033은 캐스파제 2만을 제외하고는 모든 시험된 캐스파제의 활성을 거의 완전히 억제하는 것으로 확인되었으며(도 1 참조), 이는 화학식 1의 화합물이 광범위한 캐스파제 억제활성을 보유하고 있음을 나타낸다.

<140> 실시예 3: 랫트 간세포에서 LB84033의 캐스파제 억제활성(생체내 실험)

<141> 방금 분리된 랫트 간세포를 TNF$_\alpha$ 2,000단위/㎖ 및 악티노마이신 D 100ng/㎖로 처리하여 세포의 고사를 유도하였다. 10시간 후에 세포를 회수하였다. 냉동 및 해동을 3회 반복함으로써 세포를 용해(lysis)시키고, 4℃, 12,000xg에서 20분간 원심분리함으로써 세포질 용액을 회수하였다. 세포질(단백질 ~2㎍)을 LB84033, Ac-DEVD-fmk 또는 z-VAD-CHO 100㎛M의 존재 또는 부재하에 글리세롤 20% 및 DTT 5mM을 함유하는 HEPES 완충액(100mM, pH 7.4)중의 특이 발색성 기질 200㎛M과 혼합하고 37℃에서 방치하였다. 405nm에서의 증가된 흡광도를 측정함으로써 캐스파제 활성을 에세이하였다(평균치±

SE)(n=4).

<142> 그 결과, Ac-DEVD-pNA(캐스파제-3, 7, 8 및 9), Ac-LEVD-pNA(캐스파제-4) 및 Ac-VEID-pNA(캐스파제-6)에 대해서는 강한 효소활성이 검출되었고; Ac-VDVAD-pNA(캐스파제-2)에 대해서는 보통 정도의 효소활성이 검출되었다(도 2 참조). 화학식 1의 화합물은 이들 증폭된 캐스파제 활성을 거의 완전히 억제하였으며, 그 억제활성은 펩타이드계 캐스파제 억제제인 Ac-DEVD-fmk 및 z-VAD-CHO에 필적하는 것이었다(도 2 참조). Ac-YVAD-pNA(캐스파제-1)에 대한 효소의 활성은 상대적으로 약했으나, 화학식 1의 화합물은 캐스파제-1 유사 활성의 74.1%를 억제하였다.

<143> 실시예 4: 분리된 랫트 간세포의 세포생존율 증가에 대한 LB84033의 효과(시험관내 실험)

<144> 캐스파제 억제제인 LB84033 100 μM의 존재 또는 부재하에 방금 분리된 랫트 간세포를 TNF$_\alpha$ 2,000단위/㎖ 및 악티노마이신 D 100ng/㎖로 12시간동안 처리하였다. 그 후, 크리스탈 바이올렛 염색법(n=4)으로 세포생존율을 측정하였으며, 그 결과 LB84033은 랫트의 간세포 사망을 억제하는 것으로 나타났다(평균±SE)(도 3 참조). 따라서, 이로부터 LB84033이 TNF$_\alpha$ 및 악티노마이신 D에 의해 유도된 간세포의 고사성 사망(apoptotic death)을 방지함을 확인할 수 있다.

<145> 실시예 5: 마우스에서 ConA-유발성 급성간염의 치료효과

<146> 단계 1) 혈액샘플의 제조

<147> 암컷 Balb/c 마우스(6주령, Charles River Laboratory, Osaka, Japan)를 22℃ 및 55%의 상대습도하에 12시간씩 밤/낮을 바꿔주면서 사육하였다. 이때, 사료와 물은 마

음껏 공급하였다. 발열물질(pyrogen)이 제거된 식염수에 ConA를 2.5mg/mℓ의 농도로 용해시키고 이를 꼬리정맥을 통해 상기 마우스에 ConA를 기준으로 하여 20mg/kg의 양으로 주사하였다. LB84033이 용해되어 있는 비히클(올리브오일과 10% DMSO로 구성됨) 또는 비히클 단독을 ConA 주사하기 1시간 전 및 주사한 지 4시간 후에 실험동물로 2회 복강내 주사하였다. ConA를 주사한 지 6시간 후 안와에서 혈액샘플을 얻었고, 24시간 후에 실험동물의 경부를 탈구시켜 간 및 혈액샘플을 얻었다.

<148> 단계 2: 플라스마 아미노트랜스퍼라제 활성 에세이

<149> 단계 1에서 ConA로 처리한 지 24시간 후에 회수한 혈액샘플에 대해 플라스마 AST 및 ALT 활성을 제조자의 안내서에 따라 Autokit(Youngdong Pharmaceutical Co, Seoul, Korea)를 사용하여 측정하였다. 독립된 2회의 실험을 수행하였으며, 그 결과 양실험에서 ConA가 혈청내 AST 및 ALT 활성을 급격히 상승시키며, LB84033은 용량-의존적인 방식으로 상기 상승된 효소활성을 억제하는 것으로 확인되었다(도 4A, B 참조). 두번째 실험(도 4B 참조)에서는 ConA/비히클 그룹과 ConA/LB84033 4mg/kg 그룹사이에 통계적으로 유의한 차이가 나타나지 않았는데 (AST : p=0.2972, ALT : p=0.1378), 이는 각 마우스의 효소활성이 약간씩 변화하기 때문이다. 그러나, LB84033이 20mg/kg 용량으로 투여된 경우, AST 및 ALT 활성은 ConA/비히클 그룹에 비해 명백히 감소되었다(AST : p=0.0174, ALT : p=0.0011). 첫번째 실험(도 4A 참조)에서는 LB84033이 AST 활성을 약간 증가시키는 것으로 나타났다(p=0.1033). 이러한 결과는 화학식 1의 화합물이 생체내에서 ConA에 의해 유발된 AST 및 ALT 활성의 상승을 억제하지만, 그 자신이 심각한 간독성을 일으키지는 않음을 나타낸다.

<150> 단계 3: 사이토킨 에세이

<151> 비록 대부분의 간질환 병인이 불명확하지만, 사이토킨류가 간질환에 직접적으로 관여되어 있거나 면역시스템의 활성화를 통하여 관여하는 것으로 생각되고 있다. 또한, TNF$_\alpha$, IL-1, IL-2, IL-4 및 IFN을 포함한 사이토킨류가 간질환을 증가시키는 것으로 보고되었다(참조: Chisari, F. V., 1992, *Mol. Genet. Med.* 2: 67-104; Fukuda, R. et al., *Clin. Exp. Immunolol.* 100: 446-451; Yoshioka, K. et al., *Hepatology* 10: 769-773). 따라서, 본 발명자들은 ConA에 의해 증가된 혈청 사이토킨 농도에 대한 화학식 1 화합물의 효과를 확인하기 위하여 다음과 같이 실험하였다.

<152> 단계 1에서 ConA로 처리한지 6시간 후에 회수한 혈액샘플에 대해 플라스마내의 뮤린 IL-1$_\beta$(Endogen, Inc., Boston, MA), IL-2, IL-4, IFN$_\nu$(Pharmingen, San Diego, CA) 양을 ELISA 키트를 사용하여 측정하였다. 에세이는 제조자의 안내서에 기술된 바와 동일하게 수행하였다. 각 샘플에 대해 2회 실험을 수행하였다.

<153> 실험결과, LB84033이 ConA에 의해 유발된 IL-1$_\beta$의 증가를 용량 의존적인 방식으로 억제하는 것으로 확인되었다(도 5A 참조). 한편, LB84033은 용량 의존적으로 IL-4 농도를 어느정도 감소시켰으나, 실험된 농도에서 IL-2 및 IFN$_\nu$에는 심각한 영향을 미치지 않았다(도 5B, D 참조). LB84033은 비히클을 단독으로 사용하는 경우에 비해 4가지 사이토킨 전부를 감소시켰다.

<154> 실시예 6: 조직검사 및 고사(apoptosis)의 검출

<155> 화학식 1의 화합물이 특히 간세포에서 고사-차단 효과를 나타내는지 확인하기 위하여 ConA-처리된 마우스 간에서의 고사세포의 우세정도를 하기 방법에 따라 관찰하였다.

<156> 마우스를 다양한 용량의 LB84033 존재 또는 부재하에 ConA로 처리하여[A 및 G:

ConA 및 담체 처리, B 및 H: ConA 및 LB84033 4mg/kg 처리, C 및 I: ConA 및 LB84033 20 mg/kg 처리, D 및 J: ConA 및 LB84033 100mg/kg 처리] 냉동된 간 절편을 수득하였다. 마우스를 PBS 및 담체로 처리(E 및 K)하거나, PBS 및 LB84033 100mg/kg 으로 처리(F 및 L)한 후, 역시 간 절편을 수득하였다. 절제된 마우스 간을 바로 25% 수크로오스 용액에 담그고 4℃에서 하룻밤 방치하였다. 액화질소하에서 냉동시킨 후 4㎛ 두께로 냉동절개하였다. 조직검사를 위하여 1% 파라포름알데히드 완충액으로 간 절편을 고정시키고 헤마톡실린 및 에오신으로 염색하였다(A 내지 F). 간에서의 고사세포를 관찰하기 위하여 냉동된 간 절편을 ApopTag 인사이투 고사 퍼옥시다제 검출키트(terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling : TUNEL 에세이)(Oncor, Gaithersburg, MD) 및 DAKO liquid DAB(DAKO, Carpinteria, CA)을 사용하여 염색하였다(G 내지 L). 염색은 제조자의 지시에 따라 수행하였다. 염색이 완료된 후 광학현미경을 사용하여 관찰하였다. 각 사진은 그룹당 10마리의 마우스로부터 얻은 결과중 대표적인 것이다.

<157> 실험결과, ConA는 간세포에 심각한 형태학적 및 조직학적 변화를 유발시키는 것으로 관찰되었고(도 6A 참조), 고사성 병변이 명확히 관찰되었다(도 6G 참조). 그러나, LB84033로 처리한 경우 마우스 간에서의 고사성 병변은 용량 의존적으로 감소되었고(도 6B-D 참조), LB84033의 용량을 증가시킴에 따라 세포조직이 점차로 회복되었다(도 6H-J 참조). 이러한 관찰결과는 LB84033이 치명적인 고사로부터 간세포를 보호하고 있음을 나타낸다.

<158> 실시예 7: 웨스턴 블롯팅

<159> 본 실험에서는 ConA 및 다양한 용량의 화학식 1의 화합물로 처리된 마우스로부터의 간세포 용해물중에 PARP의 분해결과 생성된 85kDa 크기의 분해물이 존재하는지를 다음과 같이 웨스턴 블롯 분석에 의해 확인함으로써 화학식 1 화합물의 고사-차단 효과를 조사하였다.

<160> ConA 또는 PBS를 다양한 용량의 LB84033과 함께 처리한 마우스로부터 회수된 간을 바로 차가운 PBS로 세척한 다음, 1% Nonidet P-40, 0.1% SDS, 1mM PMSF 및 프로테아제 억제제 칵테일 정제 Complete™(베링거 만하임, 제조자의 지시에 따라 사용)을 함유하는 차가운 3부피배량의 PBS내에 균질화시켰다. 균질화물을 얼음위에서 30분간 배양하였다. 그 후, 샘플을 16,000xg, 4℃에서 30분간 원심분리하였다. 상등액을 새 튜브에 옮기고 추가로 30분간 원심분리하였다. 회수된 용해물을 4℃에서 하룻밤동안 프로테인 G-세파로오스(Pharmacia, Uppsala, Sweden)와 함께 천천히 진탕배양하여 미리 맑은 용액으로 만들었다. 4℃, 1000xg에서 30초간 원심분리하여 상등액을 회수하고, 7% SDS-폴리아크릴아미드겔상에서 분리한 다음 니트로셀룰로오스막으로 옮겼다. 실온의 5% 탈지 건조우유 및 0.1% 트윈20을 함유하는 PBS 중에서 천천히 진탕하면서 1시간에 걸쳐 막을 블로킹하였다. 천천히 진탕하면서 1:1000으로 희석된 모노클로날 항-PARP 항체 (Pharmingen)와 함께 막을 하룻밤동안 배양하였다. PBS-트윈으로 3회 세척한 후, 블롯을 고트 항-마우스 IgG-호울스래디쉬 퍼옥시다제(1:1000 희석)와 혼성화하였다. PBS-트윈으로 3회 세척한 후 ECL 웨스턴 블롯팅 키트(Amersham-Pharmacia Biotech, San Francisco, CA)를 사용하여 시그널을 전개시킨 후 자동방사선사진 (autoradiography)로 확인하였다.

<161> 실험 결과, LB84033이 ConA에 의해 유발된 간세포의 고사성 사망에 의해 야기된

PARP 분해를 억제하는 것으로 나타났다(도 7 참조). 또한, LB84033의 고사-차단 효과는 용량 의존적인 것으로 확인되었다(도 7 참조).

<162> 실시예 8: 세포고사의 저해활성

<163> 본 발명에 따른 화학식 1 화합물의 세포고사 저해활성을 확인하기 위하여 다음과 같이 실험하였다.

<164> WI38 세포(human embryonal lung fibroblast)를 10㎝ 직경의 디쉬에서 전면(confluent)배양하였다(배지: DMEM-10% FBS). 1일째에 세포를 24웰 플레이트에 접종하였으며, 이때 배지 부피는 400㎕로 하고 밤새 배양하였다. 2일째에 감마 인터페론 200 유닛/㎖로 세포를 처리한 후, 12시간이상 배양하였다. 3일째에 DMSO중의 10mM 스톡인 시험화합물을 각각 희석하여 100㎕씩 세포에 첨가함으로써 최종농도 50, 10, 2, 0.4 ㎛M이 되도록 하였다. 이 상태로 2시간동안 배양하여 화합물이 세포내로 들어갈 시간을 준 다음, 항-Fas 항체를 40ng/웰의 농도로 처리하였다(항체를 처리한지 2시간 후 고사가 유도되며, 밤새 배양하였다). 여기에서 대조군은 Fas 항체를 처리하지 않은 경우이다. 4일째에 세포를 현미경으로 관찰한 다음, 웰당 300㎕씩 배지를 가하고 XTT working 용액 150㎕를 가한다음 2시간동안 배양하였다. 발색이 되면 상층액을 100-200㎕씩 96웰 플레이트에 옮겨서 ELISA 플레이트 리더로 A490을 측정하였다. 여기서 블랭크는 세포없이 배지만 넣은 웰에 XTT 용액을 가한 경우이다. Fas 항체로 처리하지 않은 군을 100%로 보고 나머지를 상대적으로 비교하였으며, 그 결과를 하기 표 1에 나타내었다.

<165>

【표 1】

농도(μM)	IFN	IFN+Fas Ab	Ac-DEVD-	z-DEVD-	LB84022	LB84028	LB84033
0	100	15.37					
0.4			12.16	15.13	14.44	14.67	15.51
2			14.75	16.27	18.32	17.33	64.44
10			19.16	27.38	35.37	82.40	92.68
50			35.14	47.32	78.29	90.39	100.21

<166> 상기 표 1의 결과로부터 알 수 있듯이, 감마 인터페론과 항 Fas 항체를 동시에 처리한 경우에는 15%의 세포만이 생존한 반면, 기존의 캐스파제 억제제 Ac-DEVD-CHO(reversible inhibitor) 또는 z-DEVD-cmk(irreversible inhibitor) 50μM로 처리한 경우에는 각각 35.1%, 47.3%의 세포가 생존하였고, 동일 농도의 LB84022, LB84028, LB84033으로 처리한 경우에는 각각 78%, 90%, 100%의 세포가 생존하였다. 따라서, 본 발명에 따른 캐스파제 억제제가 기존의 캐스파제 억제제에 비해 훨씬 더 강력하고 효과적인 세포고사 저해활성을 지님을 알 수 있다(도 8 참조).

【특허청구범위】

【청구항 1】

약제학적으로 허용되는 담체와 함께 유효성분으로서 캐스파제 억제제를 함유함을 특징으로 하는 소염 및 세포고사 방지용 치료제 조성물.

【청구항 2】

제1항에 있어서, 치매, 뇌졸중, AIDS로 인한 뇌손상, 당뇨, 위궤양, 간염바이러스에 의한 뇌손상, 인간 돌발성 간부전증(Fulminant hepatic failure; FHF), 패혈증(septic shock), 장기이식거부반응, 류마티스성 관절염, 또는 허혈성 심장질환에 의한 심장세포괴사에 대한 치료제 조성물.

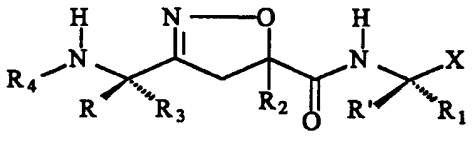
【청구항 3】

제1항에 있어서, 인간 돌발성 간부전증에 대한 치료제 조성물.

【청구항 4】

제1항 내지 제3항중의 어느 한 항에 있어서, 캐스파제 억제제가 하기 화학식 1의 화합물, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체인 조성물:

[화학식 1]



상기식에서

R 및 R' 는 각각 독립적으로 수소, 알킬(-SAC), 사이클로알킬(-SCAC), 방향족(-Ar), 또는 방향족에 의해 치환된 알킬(-SAC-Ar)을 나타내고;

R_1 은 -SAC, -SCAC, -Ar, -SAC-Ar, 또는 $-CH_2COOH$를 나타내거나, 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내며;

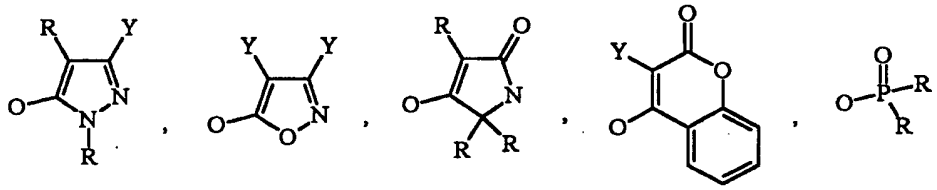
R_3 은 -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(=O)NH_2$ 또는 $-(CH_2)_2C(=O)NH_2$ 를 나타내고;

R_2 는 -H, -SAC, -SCAC, -Ar, -SAC-Ar 또는 천연 아미노산의 잔기(잔기가 카복실산 또는 염기인 경우 단순 에스테르 등으로 보호기가 결합되어 있을 수 있다)를 나타내거나, $-(CH_2)_n(O)_mR_5$ (여기서 R_5 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 0, 1, 2; m = 0, 1), $-(CH_2)_nOC(=O)R_6$ (여기서 R_6 = -SAC, -SCAC, -Ar, -SAC-Ar; n = 1, 2), 또는 $(CH_2)_n(O)_m$ Ar' (여기서 n = 0, 1, 2; m = 0, 1; Ar'=치환된 페닐 또는 이미다졸)을 나타내며;

R_4 는 모든 천연 아미노산의 유기산 아실그룹을 나타내거나, $-C(=O)R_7$ (여기서 R_7 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)OR_8$ (여기서 R_8 = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-C(=O)NR_9R_{10}$ (여기서 R_9, R_{10} = -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar), $-SOR_{11}$ (여기서 R_{11} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar), 또는 $-SO_2R_{12}$ (여기서 R_{12} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar)를 나타내고;

R_1 및 인접한 R', R_3 및 인접한 R 은 각각 함께 연결되어 $(CH_2)_n$, $(CH_2)_n$-O-$(CH_2)_m$, 또는 $(CH_2)_n$-NR_{13}-$(CH_2)_m$ [여기서 n+m <9, R_{13} = -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, 또는 -C(=O)-SAC-Ar]의 사이클릭 화합물을 형성할 수 있고;

X 는 -CN, -CHO, $-C(=O)R_{14}$ [여기서 R_{14} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)OR_{15}$ [여기서 R_{15} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-CONR_{16}R_{17}$ [여기서 R_{16} 및 R_{17} 은 각각 -H, -SAC, -SCAC, -Ar, 또는 -SAC-Ar], $-C(=O)CH_2OR_{18}$ [여기서 R_{18} 은 -SAC, -SCAC, -Ar, 또는 -SAC-Ar], 또는 $-C(=O)CH_2OC(=O)R_{19}$ [여기서 R_{19} = -SAC, -SCAC, -Ar, 또는 -SAC-Ar]을 나타내며, X 가 $-COCH_2$-W 인 경우, W 는 $-N_2$, -F, -Cl, -Br, -I, $-NR_{20}R_{21}$ 또는 $-SR_{22}$ [여기서 R_{20}, R_{21} 및 R_{22} 는 각각 독립적으로 -SAC, -SCAC, -Ar, 또는 -SAC-Ar 이거나 R_{20} 및 R_{21} 이 함께 결합하여 사이클릭 화합물을 형성할 수 있다]를 나타내거나; W 는 또한 하기 구조식을 나타낼 수 있고:



상기식에서 Y 는 -OH, OR_{23} (여기서 R_{23} = -SAC, 또는 -SCAC), $-C(=O)R_{24}$ (여기서 R_{24} = -H, -SAC, 또는 -SCAC), -F, -Cl, -Br, -I, -CN, -NC, $-N_3$, $-CO_2H$, CF_3, $-CO_2R_{25}$ (여기서 R_{25} = -SAC, 또는 -SCAC), $-C(=O)NHR_{26}$ (여기서 R_{26} = -SAC, 또는 -SCAC), 또는 $-C(=O)NR_{27}R_{28}$ (여기서 R_{27}, R_{28} = -SAC, 또는 -SCAC) 를 나타낼 수 있고, 차수(order) 및 종류에 무관하게 최대한도로 모노- 또는 폴리-치환될 수 있다.

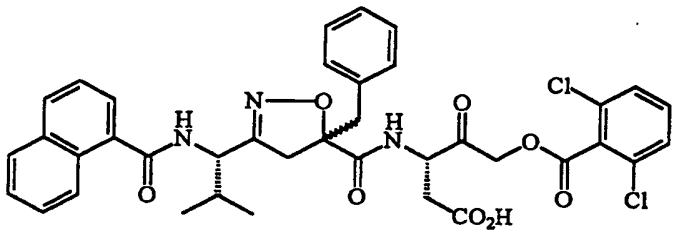
【청구항 5】

제4항에 있어서, 화학식 1의 화합물이 R 및 R' 은 수소를 나타내고; R_1 은 $-CH_2$ COOH 또는 $-CH_2COOCH_3$ 를 나타내며; R_2 는 수소, 메틸 또는 $(CH_2)_n(O)_mAr'$[n=1, 2; m=0, 1; Ar' = 치환되거나 비치환된 페닐 또는 이미다졸]을 나타내고; R_3 는 $-CH(CH_3)_2$, $-CH_2$ COOH, $-(CH_2)_2CO_2H$, $-CH_2C(O)NH_2$ 또는 $-(CH_2)_2C(O)NH_2$ 를 나타내며; R_4 는 $-C(=O)(O)_nR_{29}$

[n=0, 1; R_{29} = -Ar 또는 -SAC-Ar], $-SO_2R_{30}$[R_{30} = -Ar 또는 -SAC-Ar], 또는 $-C(=O)NHR_{31}$[R_{31} = -Ar 또는 -SAC-Ar]을 나타내고; X 는 $-C(=O)CH_2N_2$, $-C(=O)CH_2Br$, $-C(=O)CH_2Cl$, $-C(=O)CH_2OAr'$[Ar' = 페닐] 또는 $-C(=O)CH_2OC(=O)Ar''$ [Ar'' = 2,6-디클로로페닐 또는 2,6-디메틸페닐]을 나타내는 화합물인 조성물.

【청구항 6】

제4항에 있어서, 화학식 1의 화합물이 하기 구조식의 화합물, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체인 조성물.



【청구항 7】

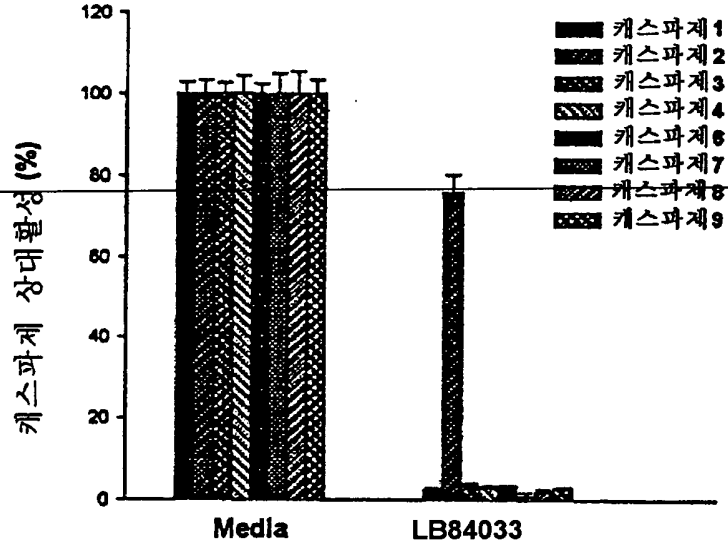
캐스파제 억제제를 사용함을 특징으로 하여 제1항에 따른 소염 및 세포고사 방지용 치료제 조성물을 제조하는 방법.

【청구항 8】

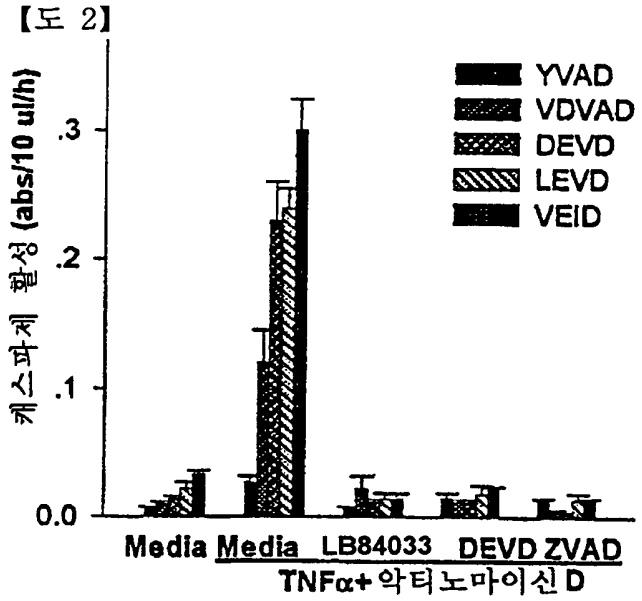
제7항에 있어서, 캐스파제 억제제가 제4항에 정의된 화학식 1의 화합물, 그의 약제학적으로 허용되는 염, 에스테르 또는 입체이성체인 방법.

【도면】

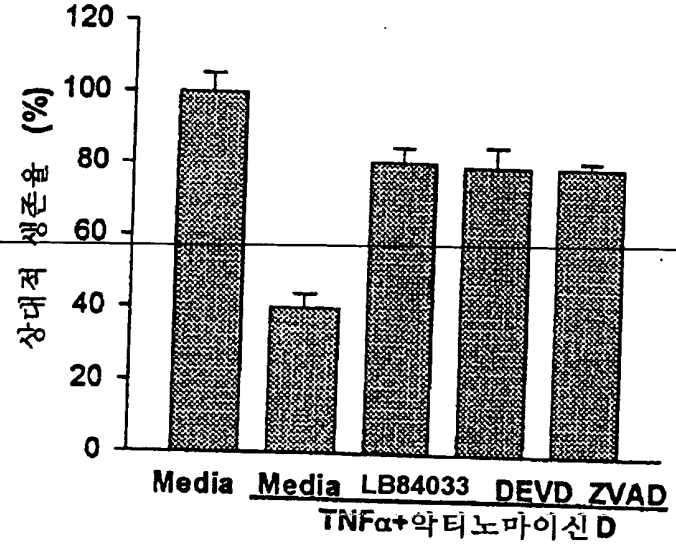
【도 1】


캐스파제 상대활성 (%)
120
100
80
60
40
20
0
캐스파제1
캐스파제2
캐스파제3
캐스파제4
캐스파제6
캐스파제7
캐스파제8
캐스파제9
Media
LB84033

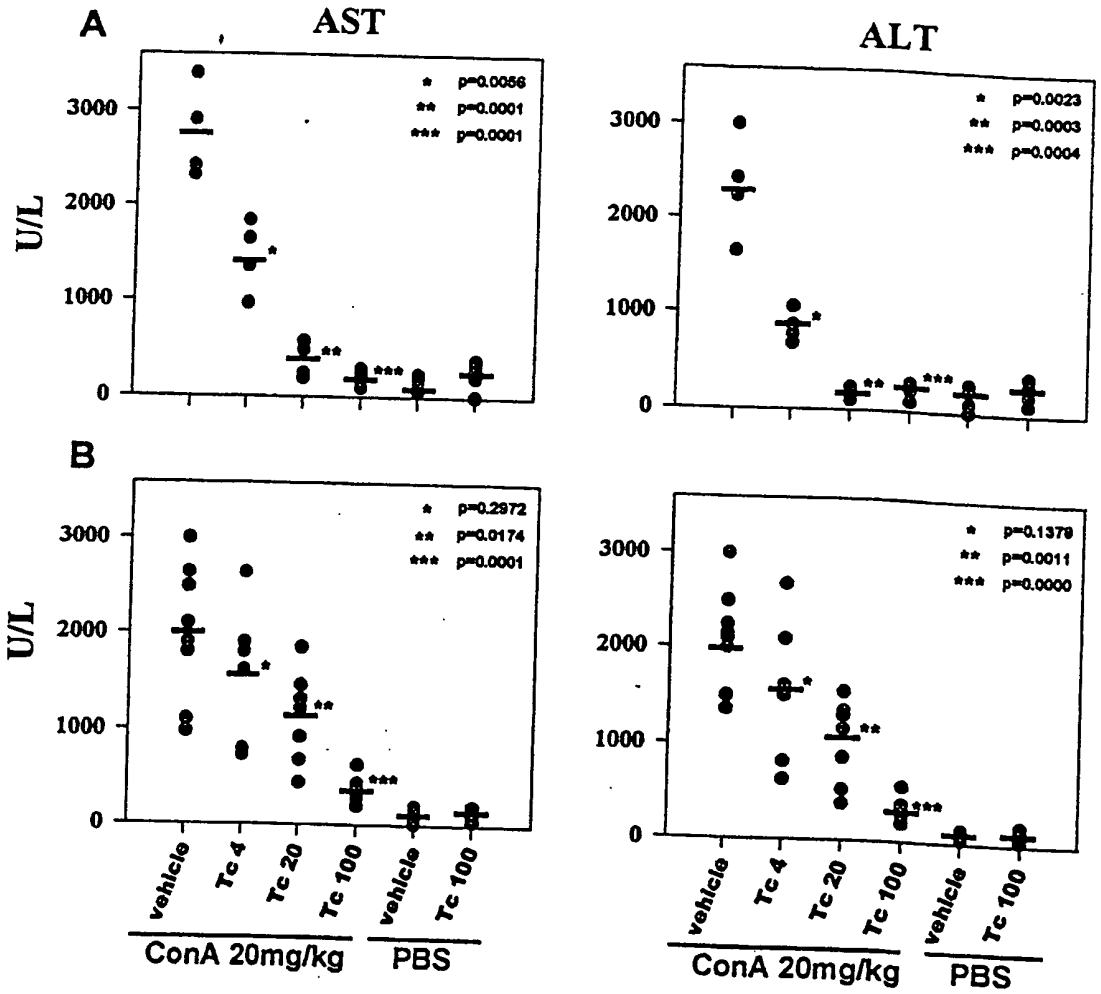
【도 2】


캐스파제 활성 (abs/10 ul/h)
.3
.2
.1
0.0
YVAD
VDVAD
DEVD
LEVD
VEID
Media Media LB84033 DEVD ZVAD
TNFα+ 악티노마이신 D

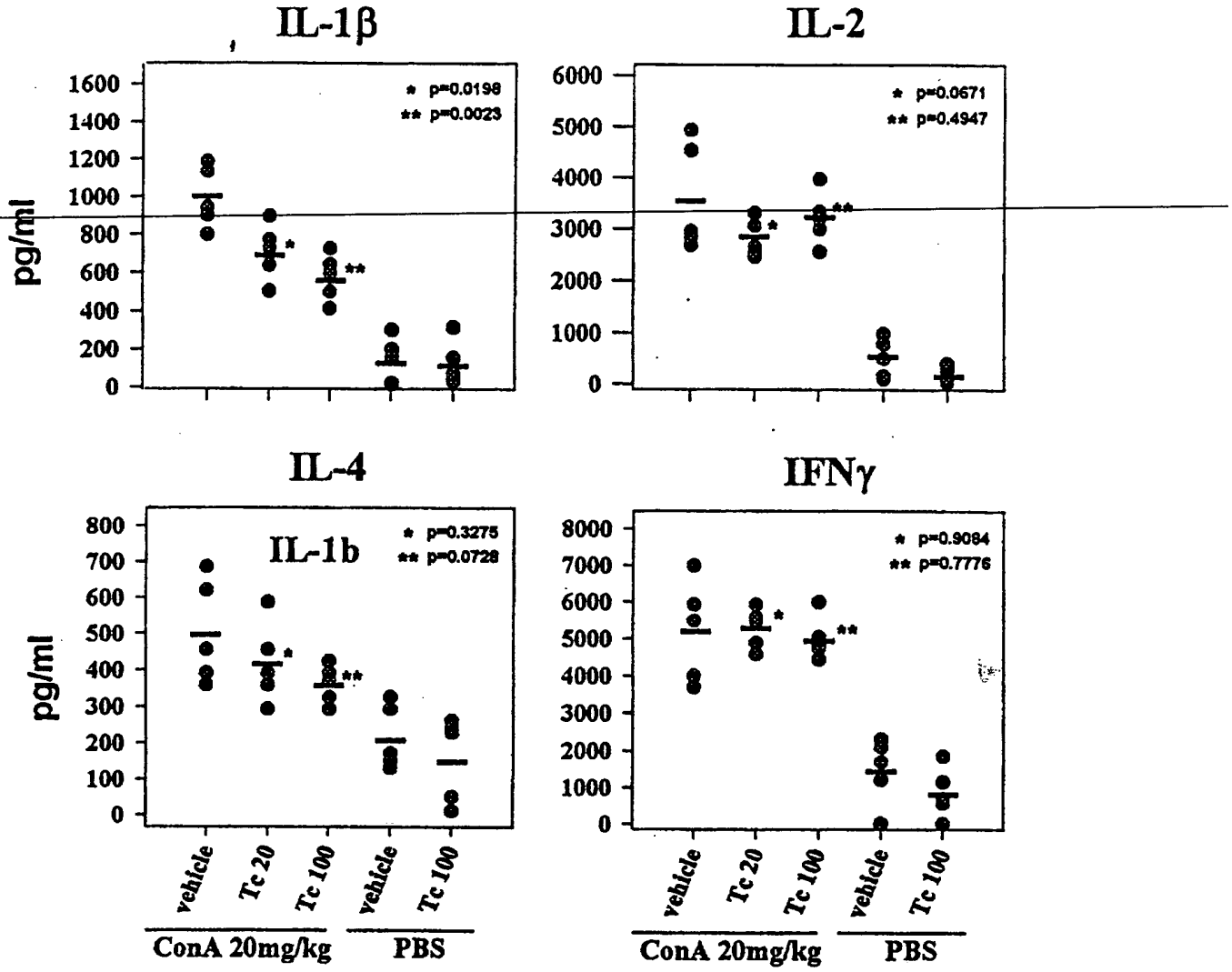
【도 3】


120
100
80
60
40
20
0
상대적 생존율 (%)
Media
Media
LB84033
DEVD
ZVAD
TNFα+악티노마이신 D

【도 4】

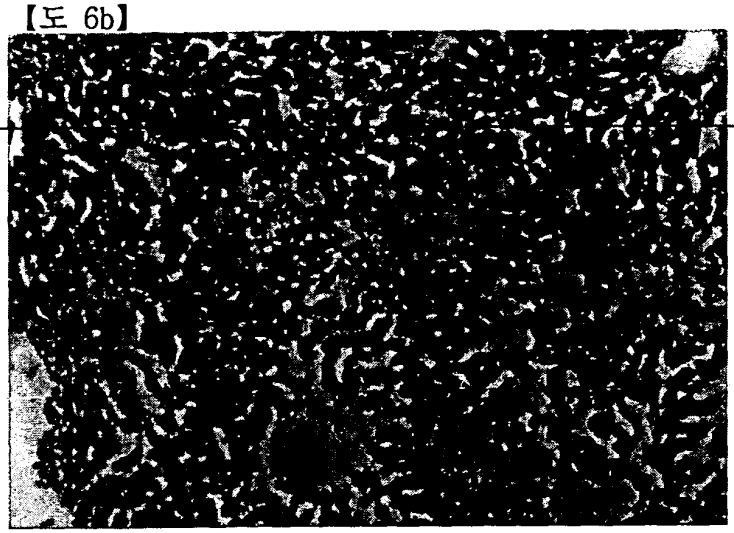

A
AST
ALT
U/L
3000
2000
1000
0
* p=0.0056
** p=0.0001
*** p=0.0001
* p=0.0023
** p=0.0003
*** p=0.0004
B
* p=0.2972
** p=0.0174
*** p=0.0001
* p=0.1379
** p=0.0011
*** p=0.0000
vehicle
Tc 4
Tc 20
Tc 100
vehicle
Tc 100
ConA 20mg/kg
PBS

【도 5】

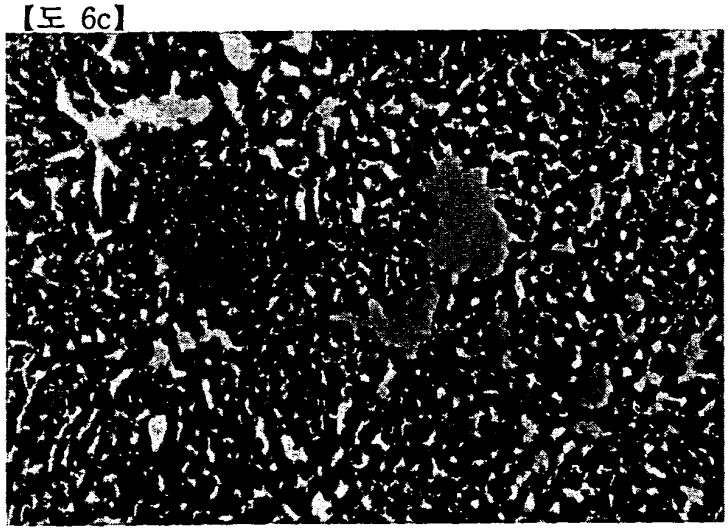

IL-1β
IL-2
IL-4
IL-1b
IFNγ
pg/ml
* p=0.0198
** p=0.0023
* p=0.0671
** p=0.4947
* p=0.3275
** p=0.0728
* p=0.9084
** p=0.7776
vehicle
Tc 20
Tc 100
vehicle
Tc 100
ConA 20mg/kg
PBS

【도 6a】

【도 6b】



【도 6c】



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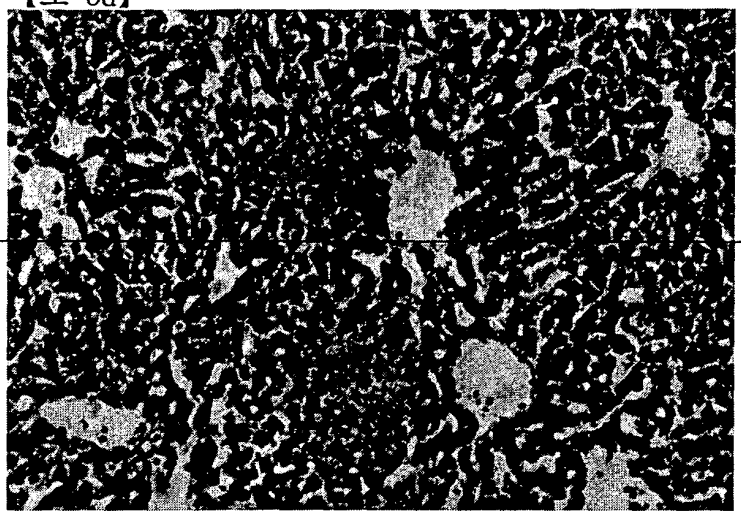
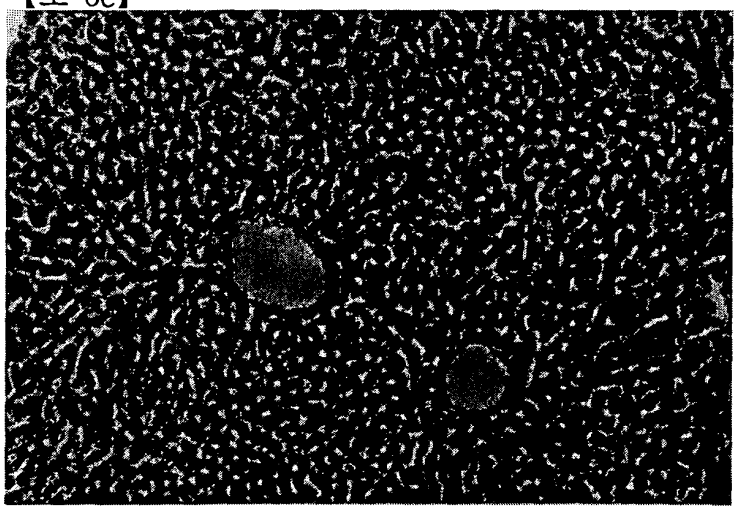
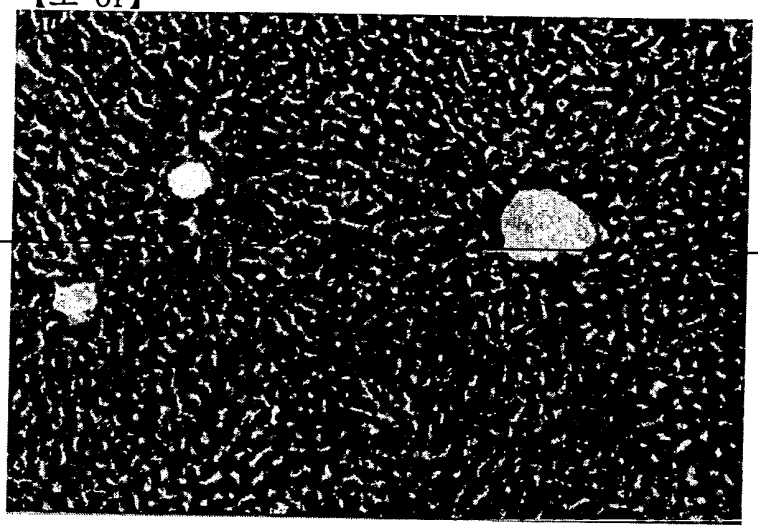


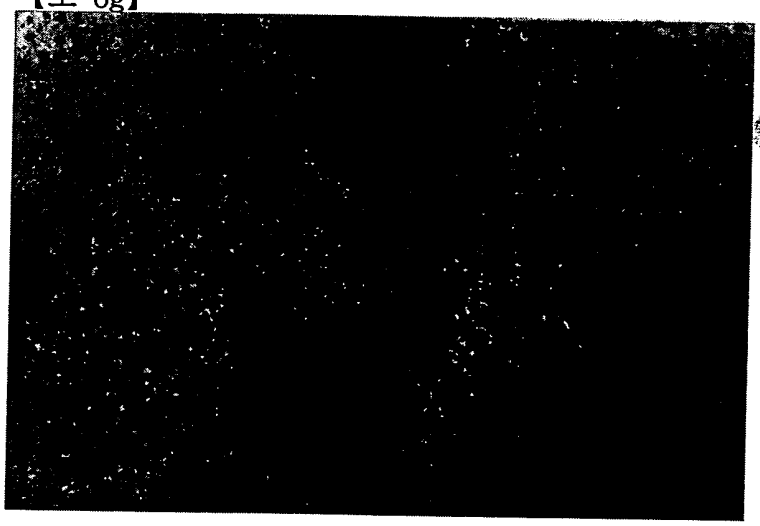
【도 6e】



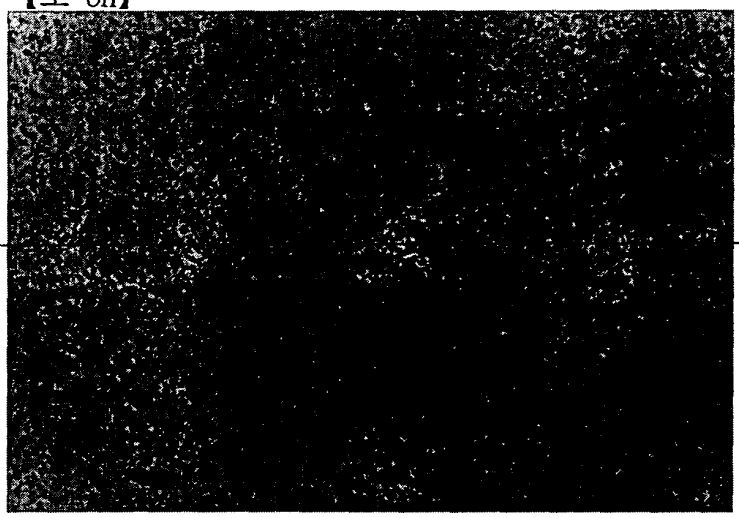
【도 6f】



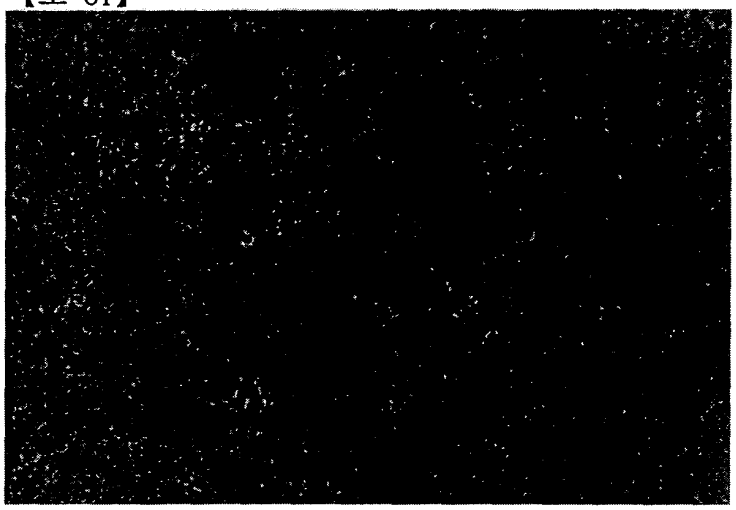
【도 6g】



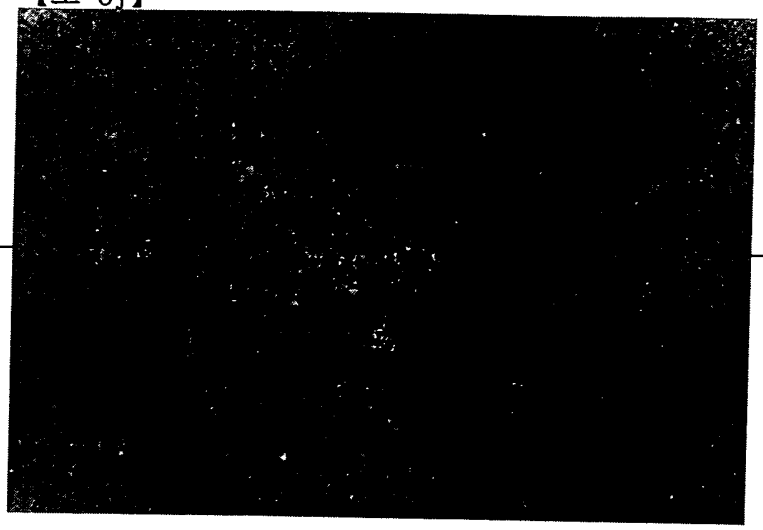
【도 6h】



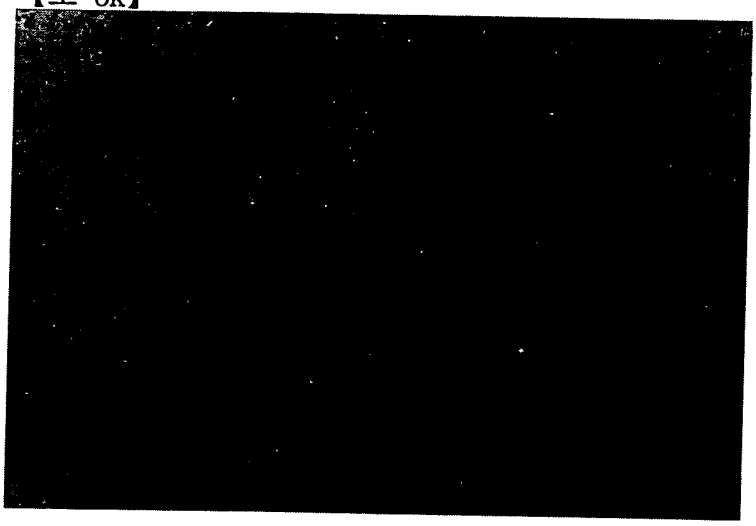
【도 6i】



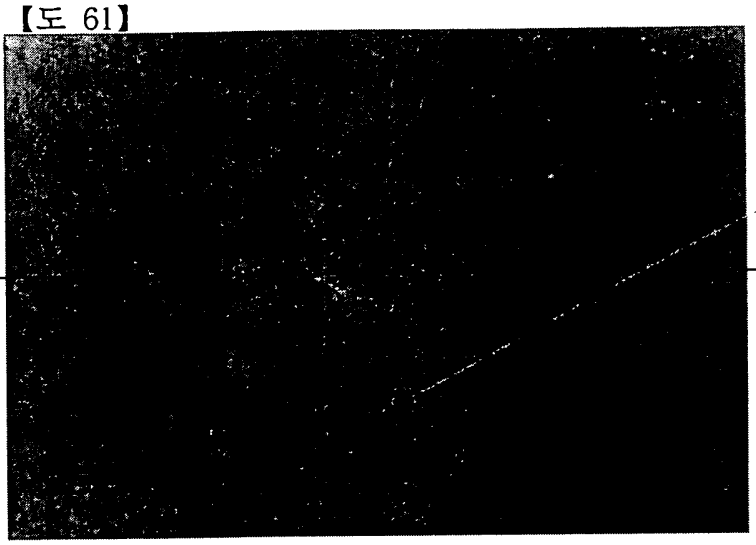
【도 6j】



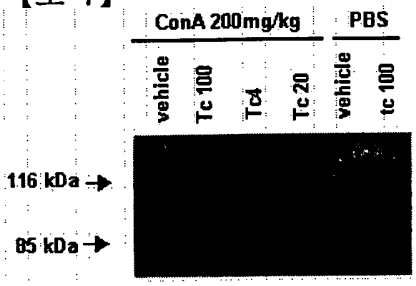
【도 6k】



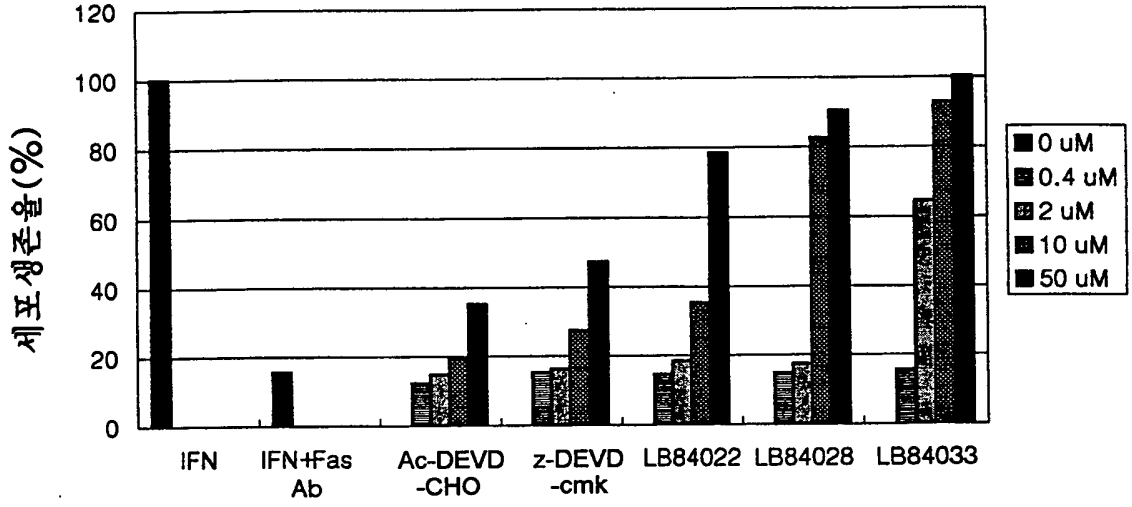
【도 61】



【도 7】


ConA 200mg/kg
PBS
vehicle
Tc 100
Tc4
Tc 20
vehicle
tc 100
116 kDa
85 kDa

【도 8】


세포생존율(%)
120
100
80
60
40
20
0
IFN
IFN+Fas Ab
Ac-DEVD -CHO
z-DEVD -cmk
LB84022
LB84028
LB84033
0 uM
0.4 uM
2 uM
10 uM
50 uM

PCT/KR 00/01047
RO/KR 18. 09. 2000.

EJU

KR00/1047

REC'D 10 OCT 2000
WIPO PCT

대 한 민 국 특 허 청
KOREAN INDUSTRIAL PROPERTY OFFICE

별첨 사본은 아래 출원의 원본과 동일함을 증명함.

This is to certify that the following application annexed hereto is a true copy from the records of the Korean Industrial Property Office.

출 원 번 호 : PCT/KR99/561
Application Number

출 원 년 월 일 : 1999년 09월 17일
Date of Application

출 원 인 : (주)엘지화학
Applicant(s)

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

2000 년 07 월 14 일

특 허 청
COMMISSIONER

PCT/KR99/00561

1999.09.17

수리관청 (박환돈)

Original (for SUBMISSION) - printed on 17.09.1999 03:27:03 PM

	ıse only ı No.	PCT/KR99/00561
0-2	International Filing Date	17 september 1999 (17.09.99)
0-3	Name of receiving Office and "PCT International Application"	Korean Industrial Property Office P C T International Application
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.84 (updated 01.07.1999)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Korean Industrial Property Office (RO/KR)
0-7	Applicant's or agent's file reference	PC99021-LG
I	Title of invention	CASPASE INHIBITOR
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	LG CHEMICAL LTD.
II-5	Address:	20, Yeido-dong, Yongdungpo-ku, 150-010 Seoul Republic of Korea
II-6	State of nationality	KR
II-7	State of residence	KR
II-8	Telephone No.	(82-042) 866-2075
II-9	Facsimile No.	(82-042) 863-2053
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	PARK, Tae-Kyo
III-1-5	Address:	LG Apt. 8-302, 381-42, Doryong-dong, Yuseong-ku, 305-340 Daejeon Republic of Korea
III-1-6	State of nationality	KR
III-1-7	State of residence	KR

III-2	**Applicant and/or inventor**	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	CHANG, Hye-Kyung
III-2-5	Address:	LG Apt. #8-204, 381-42, Doryong-dong, Yuseong-ku, 305-340 Daejeon Republic of Korea
III-2-6	State of nationality	KR
III-2-7	State of residence	KR
III-3	**Applicant and/or inventor**	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	CHUNG, Hyun-Ho
III-3-5	Address:	LG Apt. #9-205, 381-42, Doryong-dong, Yuseong-ku, 305-340 Daejeon Republic of Korea
III-3-6	State of nationality	KR
III-3-7	State of residence	KR
III-4	**Applicant and/or inventor**	
III-4-1	This person is:	applicant and inventor
III-4-2	Applicant for	US only
III-4-4	Name (LAST, First)	MIN, Chang-Hee
III-4-5	Address:	Doongji Apt. #109-1404, Doonsan-dong, Seo-ku, 302-120 Daejeon Republic of Korea
III-4-6	State of nationality	KR
III-4-7	State of residence	KR
III-5	**Applicant and/or inventor**	
III-5-1	This person is:	applicant and inventor
III-5-2	Applicant for	US only
III-5-4	Name (LAST, First)	OH, Young-Leem
III-5-5	Address:	Hyundai Apt. #104-802, Sunhwa-dong, Joong-ku, 301-050 Daejeon Republic of Korea
III-5-6	State of nationality	KR
III-5-7	State of residence	KR

III-6	**Applicant and/or inventor**	
III-6-1	This person is:	applicant and inventor
III-6-2	Applicant for	US only
III-6-4	Name (LAST, First)	PARK, Mi-Jeong
III-6-5	Address:	Expo Apt. #305-402, Junmin-dong, Yuseong-ku, 305-761 Daejeon Republic of Korea
III-6-6	State of nationality	KR
III-6-7	State of residence	KR
III-7	**Applicant and/or inventor**	
III-7-1	This person is:	applicant and inventor
III-7-2	Applicant for	US only
III-7-4	Name (LAST, First)	LEE, Tae-Hee
III-7-5	Address:	LG Apt. #7-505, 381-42, Doryong-dong, Yuseong-ku, 305-340 Daejeon Republic of Korea
III-7-6	State of nationality	KR
III-7-7	State of residence	KR
III-8	**Applicant and/or inventor**	
III-8-1	This person is:	applicant and inventor
III-8-2	Applicant for	US only
III-8-4	Name (LAST, First)	MOON, Kwang-Yul
III-8-5	Address:	Sammeri Apt. #102-304, Doonsan-dong, Seo-ku, 302-780 Daejeon Republic of Korea
III-8-6	State of nationality	KR
III-8-7	State of residence	KR
III-9	**Applicant and/or inventor**	
III-9-1	This person is:	applicant and inventor
III-9-2	Applicant for	US only
III-9-4	Name (LAST, First)	KIM, Eunice, Eun-Kyeong
III-9-5	Address:	LG Apt. #8-506, 381-42, Doryong-dong, Yuseong-ku, 305-340 Daejeon Republic of Korea
III-9-6	State of nationality	KR
III-9-7	State of residence	KR

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	CHOI, Kyu-Pal
IV-1-2	Address:	824-20, Yeoksam-dong, Kangnam-ku, 135-080 Seoul Republic of Korea
IV-1-3	Telephone No.	(82-2) 555-6888
IV-1-4	Facsimile No.	(82-2) 555-9888
IV-1-5	e-mail	HANSUNGP@chollian.net
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be		
	regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI	**Priority claim**	NONE	
VII-1	**International Searching Authority Chosen**	Austrian Patent Office (ISA/AT)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	6	-
VIII-2	Description	87	-
VIII-3	Claims	10	-
VIII-4	Abstract	1	caspase.txt
VIII-5	Drawings	0	-
VIII-7	TOTAL	104	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**		
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name (LAST, First)	CHOI, Kyu-Pal	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	

10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/AT
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT (ANNEX - FEE CALCULATION SHEET)

PC99021-LG

Original (for SUBMISSION) - printed on 17.09.1999 03:27:03 PM

(This sheet is not part of and does not count as a sheet of the international application)

0 0-1	**For receiving Office use only** International Application No.	PCT/KR99/00561
0-2	Date stamp of the receiving Office	17. 9. 1999.

0-4 0-4-1	**Form - PCT/RO/101 (Annex)** **PCT Fee Calculation Sheet** Prepared using		PCT-EASY Version 2.84 (updated 01.07.1999)	
0-9	**Applicant's or agent's file reference**		PC99021-LG	
2	**Applicant**		LG CHEMICAL LTD. , et al.	
12	**Calculation of prescribed fees**		fee amount/multiplier	total amounts (KRW)
12-1	Transmittal fee	T	⇨	45,000
12-2	Search fee	S	⇨	208,300
12-3	International fee Basic fee (first 30 sheets)	b1	528,700	
12-4	Remaining sheets		74	
12-5	Additional amount	(X)	12,200	
12-6	Total additional amount	b2	902,800	
12-7	b1 + b2 =	B	1,431,500	
12-8	Designation fees Number of designations contained in international application		82	
12-9	Number of designation fees payable (maximum 10)		10	
12-10	Amount of designation fee	(X)	122,000	
12-11	Total designation fees	D	1,220,000	
12-12	PCT-EASY fee reduction	R	-162,700	
12-13	Total International fee (B+D-R)	I	⇨	2,488,800
12-17	**TOTAL FEES PAYABLE (T+S+I+P)**		⇨	2,742,100
12-19	**Mode of payment**		cash	

VALIDATION LOG AND REMARKS

13-2-4	**Validation messages** **Priority**	Green? No priority of an earlier application has been claimed. Please verify
13-2-6	**Validation messages** **Contents**	Green? The international application contains no drawings. Please verify.
13-2-10	**Validation messages** **For receiving Office/International Bureau use only**	Green? Verify electronic data for consistency against printed form.

PCT

PC99021-LG

Original (for **SUBMISSION**) - printed on 17.09.1999 03:27:03 PM

PCT-EASY INFORMATION SHEET

(For applicant use only, DO NOT submit this sheet with the international application)

VALIDATION LOG

	Priority
Green?	No priority of an earlier application has been claimed. Please verify
	Contents
Green?	The international application contains no drawings. Please verify.
	For receiving Office/International Bureau use only
Green?	Verify electronic data for consistency against printed form.

Before submitting the International Application, please carefully verify that:

-the information contained on printed Request form is correct;
-Box IX of the Request form has been signed;
-all elements of the international application as indicated in Box VIII of the Request form have been attached; and,
-the diskette containing the PCT-EASY zip file of the International Application has been enclosed and has been clearly labeled "PCT-EASY", with the applicant's or agent's file reference, and the first applicant's name.

ATTENTION

DO NOT modify any indications on the Request form printout. The attached PCT-EASY application has been locked. If an error or an omission is discovered at this time, you must copy the submitted application as a template and make the change or correction in a new application (using the submitted application as a template). You may create such a template by copying the submitted application from the "Stored Forms" folder to the "New PCT Forms" folder. Open the new (.0WO) file created in the "New PCT Forms" folder, correct the errors and proceed with the submission process again.

Power of Attorney

Agent(Common Representative)

Name : CHOI, Kyu Pal (Patent Attorney)

Address : 824-20, Yeoksam-dong, Kangnam-ku,
Seoul 135-080, Republic of Korea

I/We, the undersigned, do hereby appoint the above-identified agent(Common representative) as my/our agent/common representative to act for me/us in proceedings concerning all of my/our International Application set forth below.

International Application No. PCT/KR99/00561

Title of Invention

CASPASE INHIBITOR

This 18th day of August, 1999

Applicant Name : LG CHEMICAL LTD.
President: SUNG, Jae Kap
Address : 20, Yoido-dong, Yongdungpo-ku,
Seoul 150-010, Republic of Korea

Power of Attorney

Agent(Common Representative)

Name : CHOI, Kyu Pal (Patent Attorney)

Address : 824-20, Yeoksam-dong, Kangnam-ku,
Seoul 135-080, Republic of Korea

I/We, the undersigned, do hereby appoint the above-identified agent(Common representative) as my/our agent/common representative to act for me/us in proceedings concerning all of my/our International Application set forth below.

International Application No. PCT/KR99/00561

Title of Invention

CASPASE INHIBITOR

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International Application No. PCT/KR99/00561

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International Application No. PCT/KR99/00561

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International Application No. PCT/KR 99 / 00561

Title of Invention

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International Application No. PCT/KR99/00561

Title of Invention

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International Application No. PCT/KR99/00561

Title of Invention

CASPASE INHIBITOR

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International Application No. PCT/KR99/00561

Title of Invention

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CASPASE INHIBITOR

Technical Field

The present invention relates to a novel isoxazoline derivative, pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof which can serve as an inhibitor for protein caspases (cysteinyl-aspartate proteinases) and a process for preparing the same and the use of the derivative as an inhibitor for caspases. The derivative according to the present invention can be effectively used in treating diseases due to caspases, such as, for example the disease in which cells are abnormally died, dementia, cerebral stroke, AIDS, diabetes, gastric ulcer, hepatic injure by hepatitis, sepsis, organ transplantation rejection reaction and anti-inflammation.

Background Art

All organisms in nature undergo the life cycle consisting of development, differentiation, growth and death. Recently, an extensive research has been made to a mechanism involved in apoptosis which would play a key role in the control of the life cycle and the outbreak of diseases. It has been reported that apoptosis is occurred by a number of factors, but largely due to the three kinds of cellular signal transport systems: the first of which is a signal transport system by the protein-protein interaction (See, Muzio M. et al., *Cell* 85, 817, 1996; Humke E.W. et al., *JBC* 273, 15702, 1998), the second, an incorporation of cytochrome C into cytoplasm via mitochondria (See, Liu X. et al., *Cell* 86:147, 1996; Li P. et al., *Cell* 91, 479, 1997), and the third, a cellular signal transport pathway by the SAPK(Stress-activated protein kinases) activation of mitogen-activation

protein kinase(MAPK) enzymes. All the pathways have been known to activate caspases cascade. As such caspases, about 10 kinds of isoenzymes have been identified in human and 14 kinds in mouse (see, Thornberry N.A. et al., *Science* 28, 1312 1998; Green D.R. *Science* 28, 1309, 1998; Ahmad M., et al., *Cancer Res.* 15, 5201 1998). The enzymes exist in the form of proenzyme which has no enzymatic activity within the cells and converted to an activated form if the cells are damaged or are exposed to a substance which leads to cellular necrosis. An activated enzyme has a heterodimer structure in which two polypeptides, i.e. larger subunits with the molecular weight of about 17-20 kDa, and smaller subunits with the molecular weight of about 10 kDa are bound together.

As present, caspases are classified into three (3) groups in view of the genetic identification analysis results and the biochemical characteristics: the first group is caspase-1, 4 and 5 which are responsible for the processing of cytokine activation, the second is caspase-3, 6 and 7 which carry out apoptosis and the third is caspase-8, 9 and 10 which are responsible for enzymatic activation in the upstream of signal transport system of apoptosis.

Among these caspases, Caspase-3 group and Caspase-8, 9, 10 etc. were recently reported to be related to apoptosis, and diseases (see, Thornberry N.A. et al., *Science*, 28, 1312, 1998).

According the recent research results, caspases are commonly activated as apoptosis is initiated, even if there is a minor difference depending upon the tissues and cells. The activated caspases then activate intracellular CAD(Caspase-activated DNAse) which finally digests intranuclear DNA to

result in cell death (Sakahira H., et al., *Nature* 1 96, 1998; Enari M et al., *Nature* 1 43, 1998). In addition, they promotes apoptosis by decomposing substrates such as PARP (Poly-ADP ribose polymerase) which is necessary for the survival of cells.

Meantime, according to the recent disease-related researches, it was reported that the activity of Caspase-3 is increased in the brain of dementia patient which promotes the production of beta amyloid peptide from beta amyloid precursor protein that is considered to be a major cause of dementia, thereby accelerating the apoptosis of brain cells (see, Kuida K. et al., *Nature* 28, 368, 1996). Further, it was reported that activation of caspases can be the direct inducer of various diseases such as sepsis (see, Haendeler J. et al., *Shock* 6, 405, 1996; Lenhoff R.J. et al., 29, 563, 1999), rheumatoid arthritis (Firestein G.S. et al., *J. Clin. Invest* 96(3), 1631, 1995), cerebral stroke (see, Hill I.E. et al., *Brain Res.*10, 398, 1995), ALS disease (see, Alexianu M.E. et al., *J. Neurochem* 63, 2365, 1994), autoimmune disease (see, Rieux-Laucat F, et al., *Science* 2, 1347, (1995), diabetes mellitus(see, Juntti-Berggren et al., *Science* 2, 86, 1993), hepatitis (Haendeler J. et al., *Shock* 6, 405, 1996), organ transplantation rejection reaction (Koglin J. et al., *Transplantation*, 27, 904, 1999; Bergese S.D. et al., *Transplantation* 27, 904, 1999), gastric ulcer (see, Slomiany B.L. et al., *J. Physiol. Pharmacol.* 96, 1631, 1995), and the like.

The researches on three dimensional structure of caspase-1 and caspase-3, catalytic mechanism of the enzyme and enzyme-substrate specificity (see, Wilson, K.P et al., *Nature* 370, 270, 1994; Walker, N.P.C. et al., *Cell* 78, 343, 1994; *Nature Struc. Biol.* 3, 619, 1996) revealed that Caspase-1 group has hydrolase-substrate specificity for the peptide sequence of (P4)-Val-X-Asp(P1) and Caspase-3 group has hydrolase-substrate specificity

for the sequence of (P4)Asp-X-X-Asp(P1).

Z-VAD-fluoromethylketon, Z-DEVD-fluoromethyl ketone which mimics the above amino acid sequence have already been used in the researches on the inhibitors and were proven to have an inhibitory activity on apoptosis of hepatic cells by an activation of caspases (see, Rodriguez I. Et al., *J. Exp. Med.*, 184, 2067, 1996; Rouquet N. et al., *Curr Biol.* 1, 1192, 1996; Kunstle G. et al., *Immunol. Lett* 55, 5, 1997), and on the apoptosis of brain cells by celebral ischemias.

However, since such peptide derivatives are deficient in drug property for clinical application, they cannot be used as therapeutics.

Disclosure of Invention

It is therefore an object of the present invention to provide a novel heterocyclic compound of the formula (I), the pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof which are useful as a caspase inhibitor.

Another object of the present invention is to provide a process for preparing the compound of formula (I).

Still another object of the present invention is to provide a pharmaceutical composition for inhibiting caspases activity which comprises as the active ingredient a therapeutically effective amount of a derivative of formula (I) and pharmaceutically acceptable carrier.

Further objects and advantages of the invention will become apparent

through reading the remainder of the specification.

The foregoing has outlined some of the more pertinent objects of the present invention. These objects should be construed to be merely illustrative of some of the more pertinent features of the invention. Many other beneficial results can be obtained by applying the disclosed invention in a different manner or by modifying the invention within the scope of the disclosure. Accordingly, other objects and a more thorough understanding of the invention may be found by referring to the detailed description of the preferred embodiment in addition to the scope of the invention defined by the claims.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the invention will be illustrated in more detail.

The present inventors have conducted an extensive research for many years in order to develop new therapeutics suitable for caspase inhibitor which has a unique structure over those known in the art. As a result, the inventors have surprisingly discovered a novel isoxazoline derivative of formula (I) which has a different structure over the known inhibitors and has excellent inhibitory activity against various substrates for caspases, and have completed the present invention.

In advance of illustrating the present invention, some important terms are defined as follows:

a) Simple Alkyl Chain (hereinafter referred to as "SAC") is meant by a carbohydrate having C_{1-8}, and contains a branched isomeric form.

b) Simple CycloAlkyl Chain (hereinafter referred to as "SCAC" is meant by a cyclic compound having C_{3-10}.

c) Aryl group (hereinafter referred to as "Ar") represents benzene [1:2,3,4,5,6], naphthalene[1,2:1,2,3,4,5,6,7,8,], pyridine [2,3,4:2,3,4,5,6], indole[1,2,3,4,5,6,7: 1,2,3,4,5,6,7], quinoline[2,3,4,5,6,7,8: 2,3,4,5,6,7,8], isoquinoline[1,3,4,5,6,7,8: 1,3,4,5,6,7,8], furan [2,3:2,3,4,5], thiophene[2,3:2,3,4,5], pyrole[1,2,3: 1,2,3,4,5], pyrimidine [2,4,5,6: 2,4,5,6], imidazole[1,2,4,5:1,2,4,5], etc. in which the former digits within the bracket represents a position where the corresponding aryl group is connected to the inhibitor according to the present invention and the latter digits after the colon represents a position where the substituent Y defined later can be attached.

Frequently referred terms are abbreviated as follows:

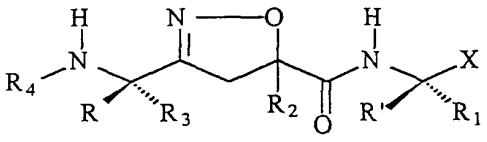
N-chlorosuccinimide : NCS
N-methylmorporline : NMM
N,N-dimethyl formamide : DMF
1-(3-dimethylaminopropyl)-3-ethylcarbodiimide: EDC
1-hydroxybenzotriazole hydrate : HOBt
Trifluoroacetic acid : TFA
t-butoxycarbonyl : Boc
benzyloxycarbonyl : Cbz
methyl : Me
ethyl : Et
equivalent : Eq or eq

The term "stereochemically isomeric forms" as used in the foregoing and

hereinafter defines all the possible isomeric forms which the derivative of formula (1) may possess. Unless otherwise mentioned or indicated, the chemical designation of compounds denotes the mixture of all possible stereochemically isomeric forms, said mixture containing all diastereomers of the basic molecular structure. Stereochemically isomeric forms of the derivatives of the formula (1) are intended to be embraced within the scope of this invention.

The pharmaceutically acceptable salts as used in the foregoing and hereinafter comprises the therapeutically active non-toxic salt forms which the derivative of formula (1) are able to form.

In an aspect, the present invention provides a novel isoxazoline derivative of the formula (I), the pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof.



(I)

In the compound of formula (I), the substituents are defined as follows.

R and R' each independently represents simple alkyl chain (-SAC), simple cycloalkyl (-SCAC), aromatic (-Ar), or simple alkyl chain substituted with aromatic (-SAC-Ar) or hydrogen, preferably represents hydrogen. Throughout the description of the specification, R' has the same meaning as R unless specifically defined.

R_1 represents -SAC, -SCAC, -Ar, or -SAC-Ar and/or contains side chain residues of natural amino acids, preferably represent $-CH_2COOH$.

R_3 represents -SAC, -SCAC, -Ar, or -SAC-Ar and/or contains side chain residues of natural amino acids, preferably represent $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(=O)NH_2$ or $-(CH_2)_2C(=O)NH_2$.

In a case where an adjacent position of R_1 or R_3 become a stereogenic center, both the stereoisomeric compounds are intended to be embraced within the scope of the present invention. Similarly, a case where two forms of compounds are co-exist (a mixture of diastereomeric compounds) is embraced within the scope of the invention. In addition, the cases where R_1 or R_3 are composed of carboxylic acids or bases with side chain residue of amino acids, their protected forms such as simple esters or pharmaceutically acceptable salt forms are also embraced within the scope of the compounds according to the invention.

R_2 represents -H, -SAC, -SCAC, -Ar, or -SAC-Ar and contains side chain residues of natural amino acids. In a case where an adjacent position due to R_2 become a stereogenic center, both the stereoisomeric compounds are embraced within the context of the compounds of the present invention, Similarly, a case where two forms of compounds are co-exist (a mixture of diastereomeric compounds) is embraced within the category of the compounds according to the invention. In addition, the cases where R_2 are composed of carboxylic acids or bases with side chain residue of amino acid, their protected forms such as simple esters or pharmaceutically acceptable salt forms are also embraced within the scope of the compounds according to the invention.

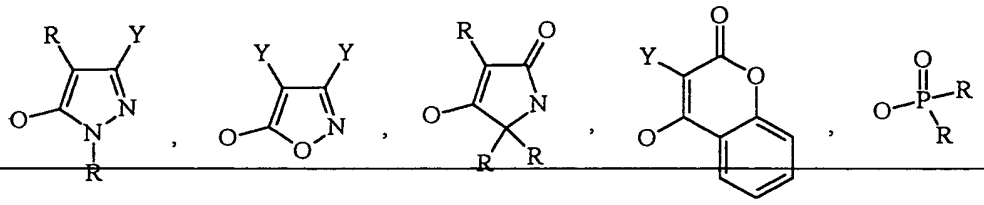
R_2 further represents $-(CH_2)_n(O)_mR_5$ (in which R_5 = -SAC, -SCAC, -Ar, -SAC-Ar; and n=0, 1, 2; m=0, 1), or $-(CH_2)_nOC(=O)R_6$ (in which R_6 =

-SAC, -SCAC, -Ar, or -SAC-Ar; and n=1, 2). Preferable R_2 represents $(CH_2)_n(O)_mAr'$ (in which n=0, 1, 2; m = 0, 1; Ar' = substituted phenyl or imidazole), methyl or hydrogen.

R_4 represents an organic acid acyl group of all the natural amino acids or represents $-C(=O)R_7$ (in which R_7 = -SAC, -SCAC, -Ar, or -SAC-Ar), $-C(=O)OR_8$ (in which R_8 = -SAC, -SCAC, -Ar, or -SAC-Ar), $-C(=O)NR_9R_{10}$ (in which R_9, R_{10} = -H, -SAC, -SCAC, -Ar, or -SAC-Ar), $-SOR_{11}$ (in which R_{11} = -SAC, -SCAC, -Ar, or -SAC-Ar), or $-SO_2R_{12}$ (in which R_{12} = -SAC, -SCAC, -Ar, or -SAC-Ar).

In cases where R_1 and the adjacent R', and/or R_3 and the adjacent R are connected to each other to form a cyclic compound, R_1-R' or R_3-R together represents $(CH_2)_n$, $(CH_2)_n$-O-$(CH_2)_m$, or $(CH_2)_n$-NR_{13}-$(CH_2)_m$ [in which n+m <9, R_{13} = -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, or -C(=O)-SAC-Ar].

X represents -CN, -CHO, $-C(=O)R_{14}$ [in which R_{14} = -SAC, -SCAC, -Ar, or -SAC-Ar], $-C(=O)OR_{15}$ [in which R_{15} = -SAC, -SCAC, -Ar, or -SAC-Ar], $-CONR_{16}R_{17}$ [in which R_{16} and R_{17} each represents -H, -SAC, -SCAC, -Ar, or -SAC-Ar], $-C(=O)CH_2OR_{18}$ [in which R_{18} represents -SAC, -SCAC, -Ar, or -SAC-Ar], or $-C(=O)CH_2OC(=O)R_{19}$ [in which R_{19} = -SAC, -SCAC, -Ar, or -SAC-Ar]. The invention further encompasses a case where if X represents $-COCH_2$-W, W represents $-N_2$, -F, -Cl, -Br, -I, $-NR_{20}R_{21}$ or $-SR_{22}$ [in which wherein R_{20}, R_{21} and R_{22} each independently represents -SAC, -SCAC, -Ar, or -SAC-Ar or a case where R_{20} and R_{21} are connected to form a cyclic compounds]. W also represents



in which Y represents -OH, OR_{23} (in which R_{23} = -SAC, or -SCAC), $-C(=O)R_{24}$ (in which R_{24} = -H, -SAC, or -SCAC), -F, -Cl, -Br, -I, -CN, -NC, $-N_3$, $-CO_2H$, CF_3, $-CO_2R_{25}$ (in which R_{25} = -SAC, or -SCAC), $-C(=O)NHR_{26}$ (in which R_{26} = -SAC, or -SCAC), and $-C(=O)NR_{27}R_{28}$ (in which R_{27}, R_{28} = -SAC, or -SCAC) and can be mono- or poly-substituted at its maximum regardless of the order and the kinds.

Among the compound of formula (I), preferred are those in which

a) R and R' represent hydrogen,

b) R_1 represents $-CH_2COOH$,

c) R_2 represents $(CH_2)_n(O)_mAr'$ [in which n=1, 2; m=0, 1; Ar' = substituted phenyl or imidazole], methyl or hydrogen,

d) R_3 represents $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(O)NH_2$ or $-(CH_2)_2C(O)NH_2$,

e) R_4 represents $-C(=O)(O)_nR_{29}$ [in which n=0, 1; R_{29} = -Ar, or -SAC-Ar], $-SO_2R_{30}$ [in which R_{30} = -Ar, or -SAC-Ar], or $-C(=O)NHR_{31}$, [in which R_{31} = -Ar, or -SAC-Ar],

f) X represents $-C(=O)CHN_2$, $-C(=O)CH_2Br$, $-C(=O)CH_2Cl$, $-C(=O)CH_2OAr''$ [Ar'' = preferably phenyl] or $-C(=O)CH_2OC(=O)Ar'''$ [in which Ar''' = preferably 2,6-dichlorophenyl or 2,6-dimethylphenyl].

Most preferred compounds are selected from the group consisting of (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxy methyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid;

(2S)-2-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxy methyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 1-(N-methyl-N-methoxy)-amide;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro -isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro -isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro -isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-1-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic

acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(quinoline-2-yl-carbonylamino)-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-sulfonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-naphthyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(2S)-2-acetylamino-succinoylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-4,5-dihydro-

isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (diasteromeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid

(diastereomeric mixture);
(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid (diastereomeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid (diastereomeric mixture);
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-

pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-(1-

imidazolyl-methyl)-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-(succinoylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(succinoylamino)-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid; and

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-piperidinyl)-pentanoic acid.

In an another aspect, the present invention provides a process for preparing a compound of formula (I).

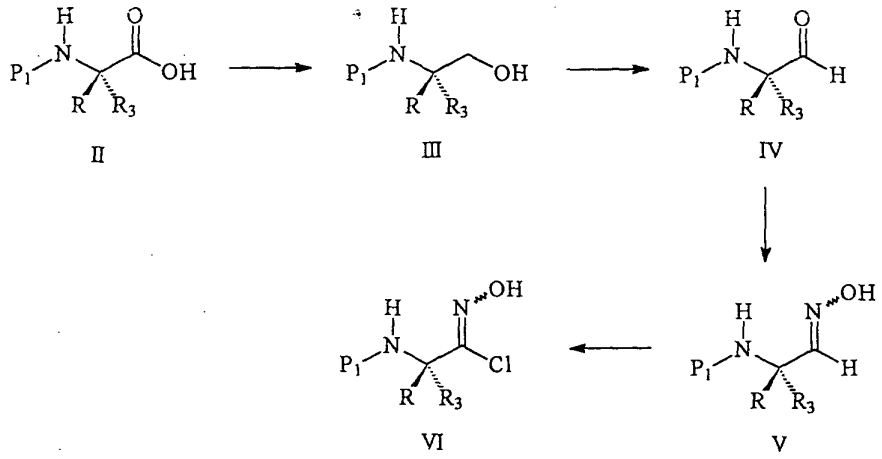
Hereinbelow, a process for preparing the isoxazoline derivatives of formula (I) according to the present invention will be explained with respect to Reaction Schemes 1 and 2. It should be understood that the reaction schemes generally illustrate the specific process used in the present invention, but any modification of the unit operations may be made without departure of the spirit of the invention. Therefore, the present invention should not be limited to the following preferred embodiments.

In the first step, amino protected amino acid (II) (commerically available from Novabiochem) is reduced to give N-protected amino alcohol (III) which is then oxidized to give N-protected amino aldehyde (IV).

N-protected amino aldehyde (IV) is reacted with hydroxylamine-hydrochloride and sodium carbonate in a mixed solution of an alcohol and

water to give an oxime (V) (syn- and anti-oxime). The resulting oxime derivative (V) is treated with NCS (N-chlorosuccinimide) in an aqueous solution of dimethylformamide to give hydroxamoyl chloride (VI). As the representative substituents used in the synthesis of hydroxamoyl chloride, the following groups may be exemplified: P_1 represents Cbz, t-Boc, Fmoc, Teoc(trimethylsilyl-ethyloxycarbonyl), etc.; R represents H and R_3 represents $-CH_2CH_2CO_2Bu(t)$, $-CH_2CO_2Me$, $-CH_2CO_2Bu(t)$, -isopropyl, phenylmethyl, and the like.

Reaction Scheme 1



In the above Reaction Scheme 1, the following combinations of a) to g) for the commercially available compounds (II) to (VI) may be synthesized.

a) P_1 = Cbz, R = H, R_3 = i-Pr

b) P_1 = t-Boc, R = H, R_3 = i-Pr

c) P_1 = Fmoc, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$

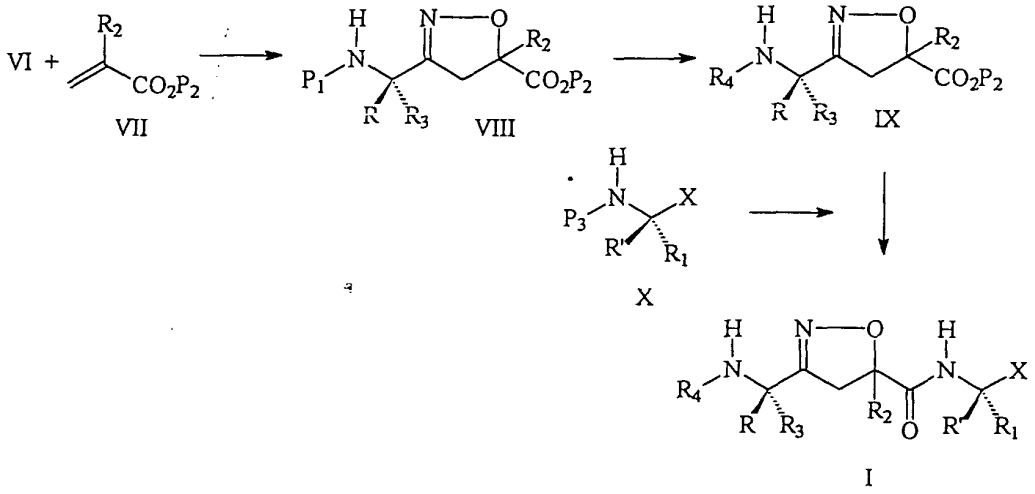
d) P_1 = t-Boc, R = H, R_3 = CH_2CO_2Me

e) P_1 = Cbz, R = H, R_3 = $CH_2CO_2Bu(t)$

f) P_1 = Fmoc, R = H, R_3 = $CH_2CO_2Bu(t)$

g) P_1 = Boc or Cbz, R = H, R_3 = CH_2Ph

Reaction Scheme 2



In the second step, the hydroxamoyl chloride (VI) thus obtained is then reacted with acrylate derivative (VII) to give isoxazoline derivative (VIII). If necessary, isoxazoline derivative (VIII) may be synthesized directly from the oxime derivative (V).

If a compound having the protecting group P_1 can be used as the inhibitor (for example, P_1 is a Cbz group), the isoxazoline derivative (VIII) is directly reacted with the compound (X) to give a compound of formula (I), and if it is necessary to convert the protecting group P_1 into other substituent, P_1 is removed and R_4 is introduced thereinto.

In the above Reacion Scheme 2, the following combination of substituents

may be synthesized.

In the compound (VIII),

a) P_1 = Cbz, R = H, R_3 = i-Pr, R_2 = H, P_2 = Et

b) P_1 = Cbz, R = H, R_3 = i-Pr, R_2 = H, P_2 = H

c) P_1 = Cbz, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = Et

d) P_1 = Cbz, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = H

e) P_1 = Fmoc, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, P_2 = CH_3(or Et)

f) P_1 = Teoc, R = H, R_3 = i-Pr, R_2 = CH_3, P_2 = H

g) P_1 = t-Boc, R = H, R_3= i-Pr, R_2= $PhCH_2$, P_2=Et

h) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, P_2 = Et

i) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = 1-naphthyl, P_2 = Et

j) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = 2-naphthyl, P_2 = Et

k) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = phenyl, P_2 = Et

l) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = 4-bromophenyl, P_2 = Et

m) P_1 = t-Boc, R = H, R_3 = i-Pr, R_2 = $AcOCH_2$, P_2 = Et

In the compound (IX),

a) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, P_2 = Et

b) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, P_2 = H

c) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = Et

d) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = H

e) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = Et

f) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = H

g) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = Et

h) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = CH_2OPh, P_2 = H

i) R_4 = 2-naphthoyl, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, P_2 = CH_3

j) R_4 = 2-naphthoyl, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, P_2 = H

k) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

l) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

m) R_4 = 2-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, P_2 = Et

n) R_4 = 2-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, P_2 = H

o) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, P_2 = Et

p) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, P_2 = H

q) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = H, P_2 = Et

r) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = H, P_2 = H

s) R_4 = hydrocinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

t) R_4 = hydrocinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

u) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

v) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

w) R_4 = 1-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

x) R_4 = 1-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

y) R_4 = 3-indoleacetyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

z) R_4 = 3-indoleacetyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

aa) R_4 = 3-indolepropionyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

ab) R_4 = 3-indolepropionyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

ac) R_4 = *trans*-cinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

ad) R_4 = *trans*-cinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

ae) R_4 = phenylmethylsulfonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

af) R_4 = phenylmethylsulfonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

ag) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = H, P_2 = Et

ah) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = H, P_2 = H

ai) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = Et

aj) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, P_2 = H

ak) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = 1-imidazolyl, P_2 = Et

al) R_4 = 1-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = 1-imidazolyl, P_2 =

H

am) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, P_2 = CH_3

an) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, P_2 = H

ao) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = i-Pr, R_2 = CH_3, P_2 = CH_3

ap) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = i-Pr, R_2 = CH_3, P_2 = H

In the compound (X),

a) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =CO_2Me

b) P_3 = HCl+H, R = H, R_1=$CH_2CO_2Bu(t)$, X =CO_2Me

c) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =$COCH_2N_2$

d) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =$COCH_2Br$

e) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =$COCH_2OPh$

f) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =$CH(OH)CH_2OPh$

g) P_3 = H, R = H, R_1=$CH_2CO_2Bu(t)$, X =$CH(OH)CH_2OPh$

h) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$,
X = $CH(OH)CH_2OC(O)Ph$(2,6-dichloro)

i) P_3 = H, R = H, R_1=$CH_2CO_2Bu(t)$,
X =$CH(OH)CH_2OC(O)Ph$(2,6-dichloro)

j) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =CONMe(OMe)

k) P_3 = H, R = H, R_1=$CH_2CO_2Bu(t)$, X =CONMe(OMe)

l) P_3 = Cbz, R = H, R_1=$CH_2CO_2Bu(t)$, X =$CH(OH)CH_2O$- (1-naphthyl)

m) P_3 = H, R = H, R_1=$CH_2CO_2Bu(t)$, X =$CH(OH)CH_2O$- (1-naphthyl)

In the compound (I),

a) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = CO_2H

b) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$,

X = $C(=O)CH_2N_2$

c) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2Br$

d) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

e) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph$ (2,6-dichloro)

f) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = 2-naphthyloxymethylcarbonyl

g) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = 1-naphthyloxymethylcarbonyl

h) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $CH(OH)CH_2OPh$

i) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

j) R_4 = 2-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $CH(OH)CH_2OPh$

k) R_4 = 2-naphthalenesulfonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

l) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $CH(OH)CH_2OPh$

m) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $CH(OH)CH_2OPh$

n) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2N_2$

o) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2Br$

p) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

q) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)$-Ph-(2,6-dichloro)

r) R_4 = N-acetyl-β-t-butyl aspartyl, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, R_1 = $CH_2CO_2Bu(t)$, X = $CH(OH)CH_2OPh$

s) R_4 = N-acetyl-β-t-butyl aspartyl, R = H, R_3 = $CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

t) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)NMe(OMe)$

u) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_3$

v) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = CO_2CH_3

w) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = CO_2H

10x) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2N_2$

y) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2Br$

z) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)$-Ph-2,6-dichloro

aa) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)NMe(OMe)$

ab) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = $PhOCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_3$

ac) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = CO_2CH_3

ad) R_4 = Cbz, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph$(2,6-dichloro)

ae) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

af) R_4 = hydrocinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

ag) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

ah) R_4 = 1-naphthalenesulphonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

ai) R_4 = 3-indoleacetyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

aj) R_4 = 3-indolepropionyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

ak) R_4 = *trans*-cinnamoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

al) R_4 = phenylmethylsulfonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

am) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

an) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

ao) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OC(=O)Ph(2,6\text{-dichloro})$

ap) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

aq) R_4 = 2-quinolinecarbonyl, R = H, R_3 = i-Pr, R_2 = 1-imidazolyl, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OCPh$

ar) R_4 = 2-naphthoyl, R = H, R_3 = i-Pr, R_2 = H, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_3$

as) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = $(CH_2CH_2CO_2Bu(t)$, R_2 = CH_3, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

at) R_4 = $COCH_2CH_2CO_2Bu(t)$, R = H, R_3 = i-Pr, R_2 = CH_3, R_1 =

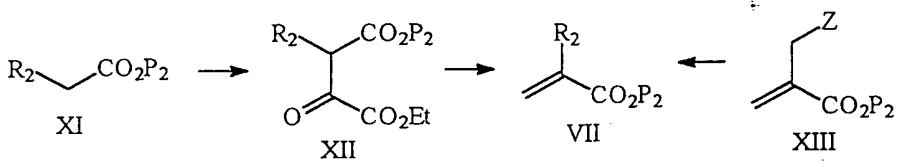
$CH_2CO_2Bu(t)$, X = $C(=O)CH_2OPh$

au) R_4 = 1-naphthoyl, R = H, R_3 = i-Pr, R_2 = $PhCH_2$, R_1 = $CH_2CO_2Bu(t)$, X = $C(=O)CH_2N(CH_2)_5$

In Reaction Scheme 2, the functional group X of compound (X) may be introduced by several unit operations after the reactions involved in the synthesis of the compound (VIII) or (IX), or the compound (VIII) or (IX) already having desired substituent X may be proceed with the subsequent reactions.

The acrylate derivative (VII) may be synthesized by any of two processes as depicted in Reaction Scheme 3 below.

Reaction Scheme 3



Ester derivative (XI) is reacted with diethyl oxalate to give oxalate derivative (XII) which is then reacted in the presence of a base to give desired acrylate derivative (VII). Alternatively, it may be synthesized by various process starting from the known compound (XIII). That is, the known compound (XIIIa) is easily converted into compounds (XIIIb), (VIIe), (VIIf), (VIIg), etc.

In the compounds (XI) and (XII), the substituents are examplified as follows:

a) P_2 = Et, R_2 = Ph

b) P_2 = Et, R_2 = 4-bromophenyl

c) P_2 = Et, R_2 = 1-naphthyl

d) P_2 = Et, R_2 = 2-naphthyl

~~In the compounds (VII) and (XIII), the following combination of the~~ substituents can be synthesized by the above process.

In the compound of (VII),

a) R_2 = Ph, P_2 = Et

b) R_2 = 4-bromophenyl, P_2 = Et

c) R_2 = 1-naphthyl, P_2 = Et

d) R_2 = 2-naphthyl, P_2 = Et

e) R_2 = CH_2OAc, P_2 = Et

f) R_2 = CH_2Ph, P_2 = Et

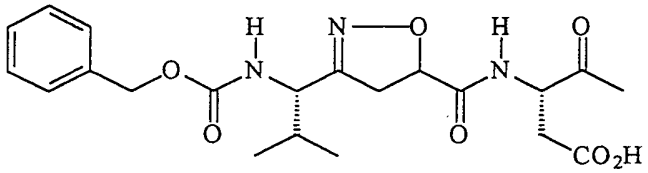
g) R_2 = CH_2OPh, P_2 = Et

In the compound (XIII),

a) P_2 = Et, Z = OH

b) P_2 = Et, Z = Br

Hereinafter, the representative compounds synthesized by the process of the invention will be listed with respect to their structural formulae. However, they are presented for the purpose of illustration of the synthesis of the compounds of the invention and for substantiating the fact that the compounds of the invention can be synthesized by the above mentioned preparation process, but the present invention should not be limited to the compounds listed in any manner.

(1) 

Diastereomer

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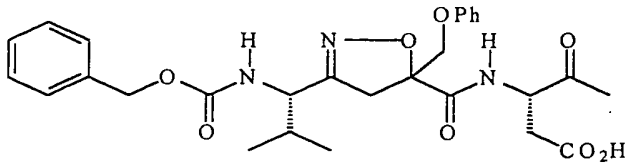
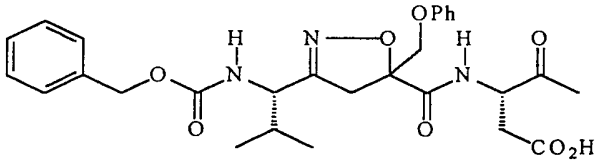
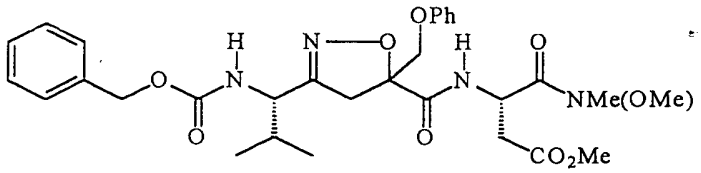
(2) 

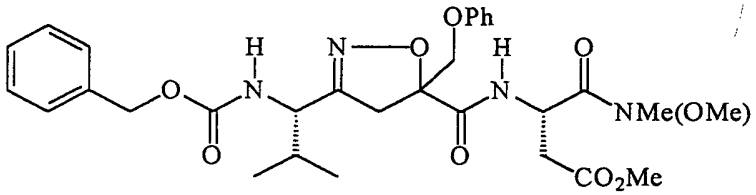
Diastereomer
(less polar)

(3) 

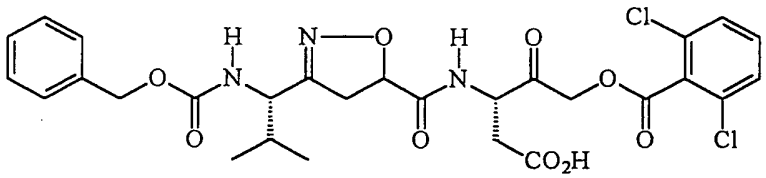
Diastereomer
(more polar)

(4) 

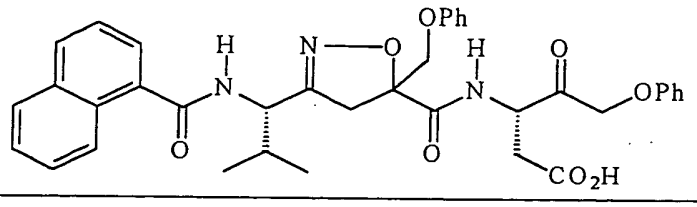
Diastereomer
(less polar)

(5) 

Diastereomer
(more polar)

(6) 

Diastereome

(7)  Diastereomer

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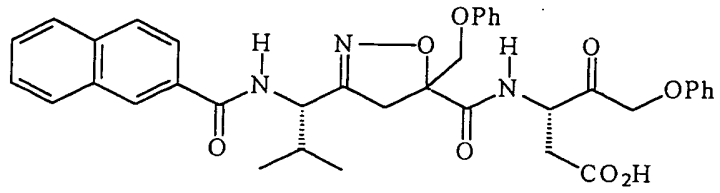
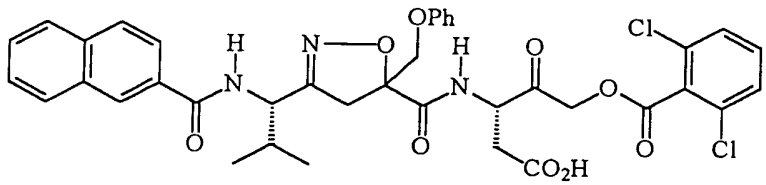
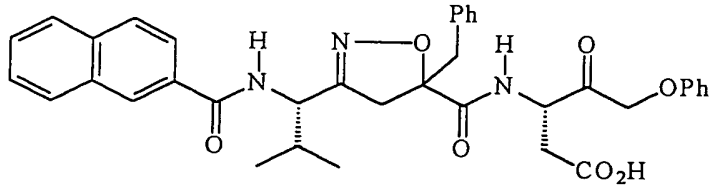
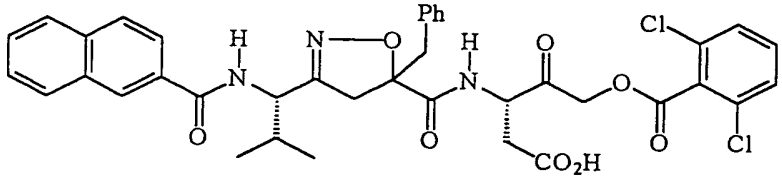
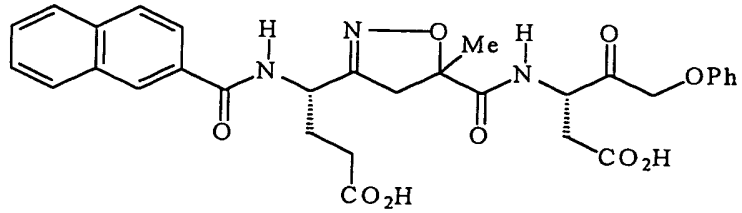
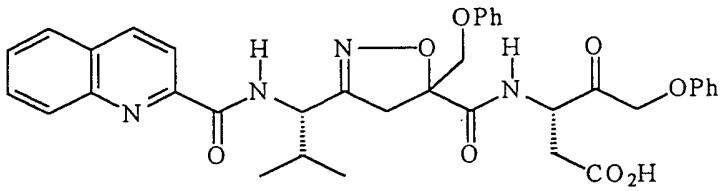
(8)  Stereoisomer

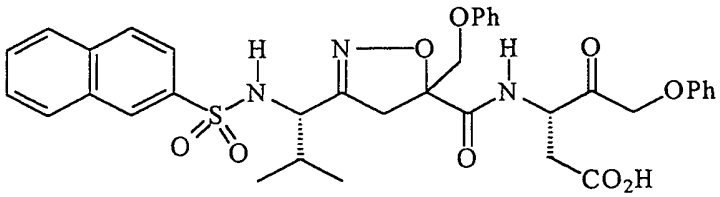
(9)  Stereoisomer

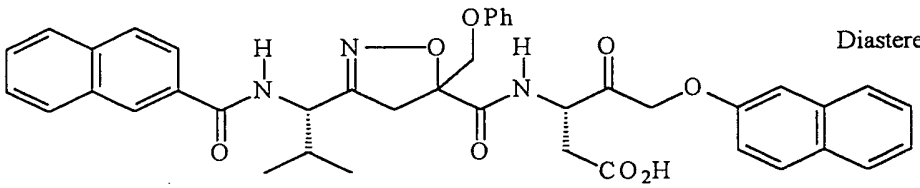
(10)  Stereoisomer

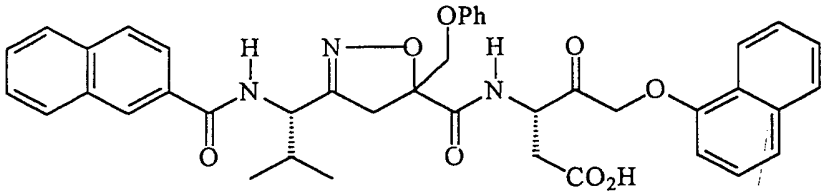
(11)  Stereoisomer

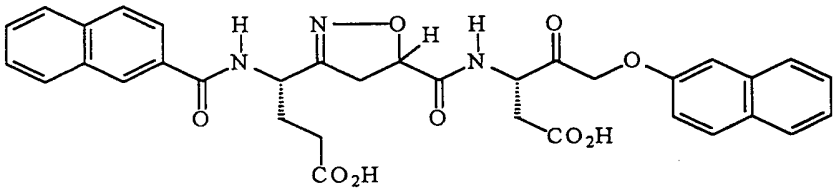
(12)  Diastereomer

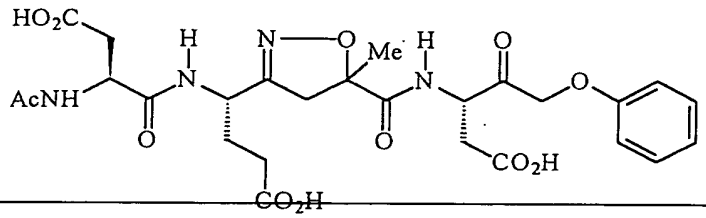
(13)  Diastereomer

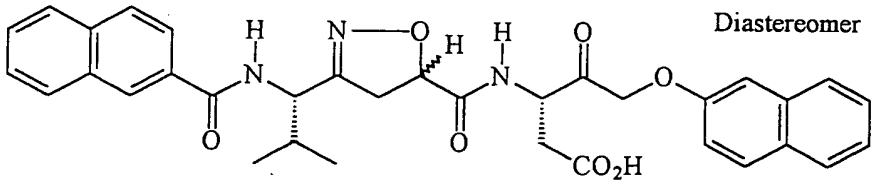
(14)  Diastereomer

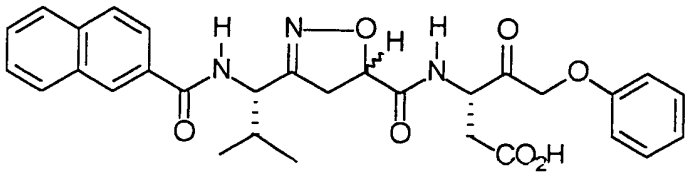
(15)  Diastereomer

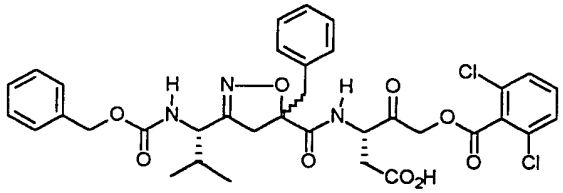
(16)  Diastereomer

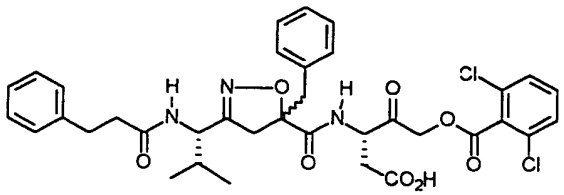
(17)  Diastereomer

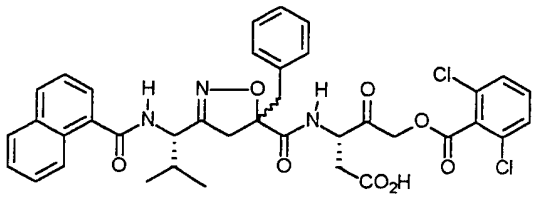
(18)  Diastereomer

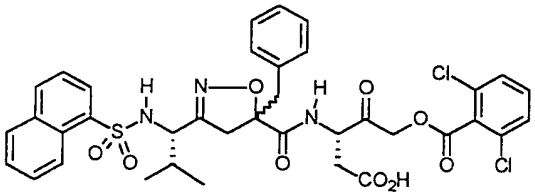
(19)  Diastereomer

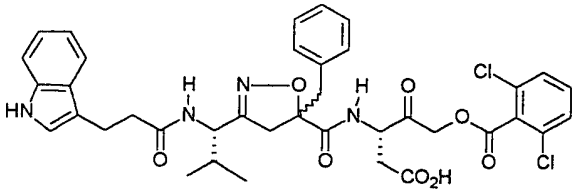
(20) 

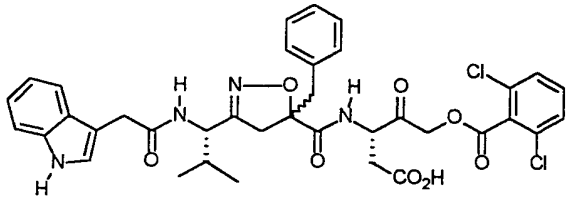
(21) 

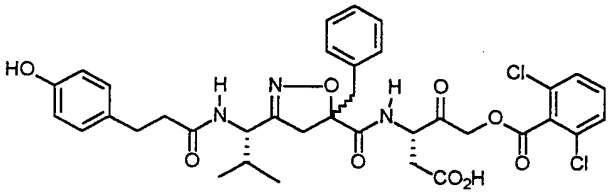
(22) 

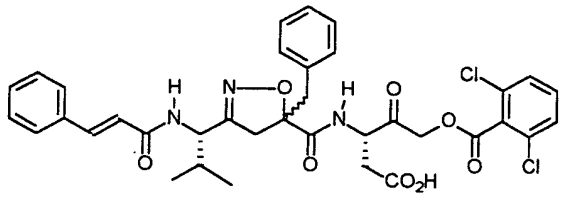
(23) 

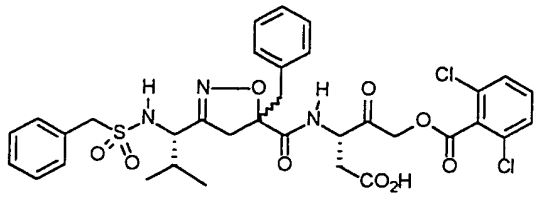
(24) 

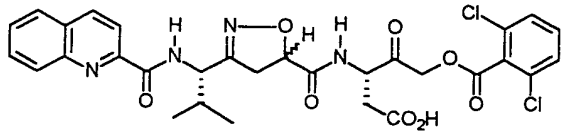
(25) 

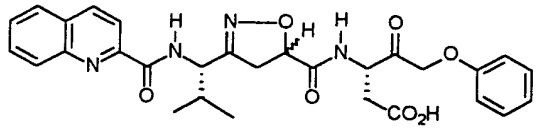
(26) 

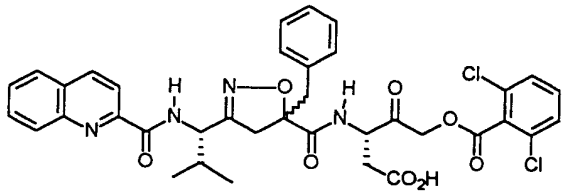
(27) 

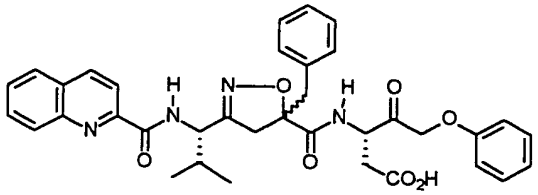
(28) 

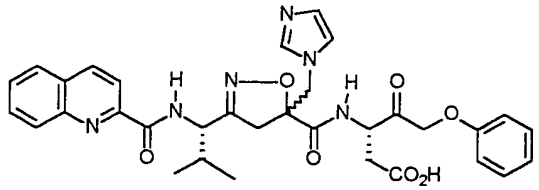
(29) 

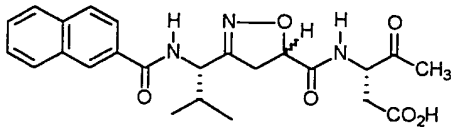
(30) 

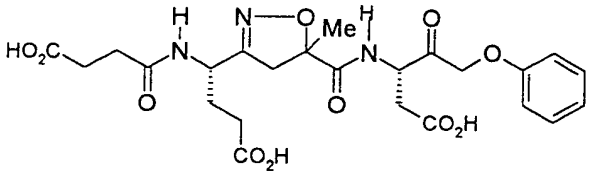
(31) 

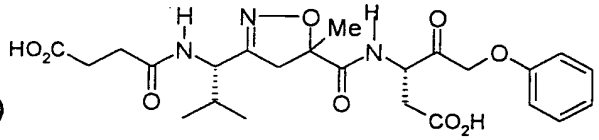
(32) 

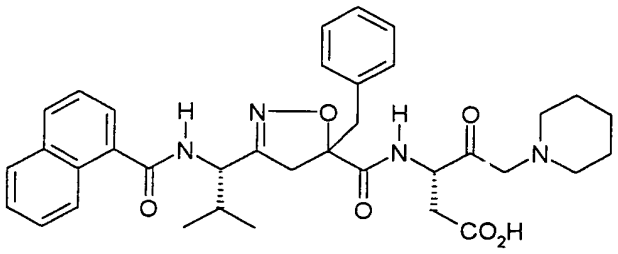
(33) 

(34) 

(35) 

(36) 

(37) 

(38) 

The isoxazoline derivative of formula (I) and the pharmaceutically

acceptable salts, the esters, and the isomers thereof have useful pharmacological properties. For example, the derivative of the formula (I) posses an inhibitory activity for caspases. Due to their pharmacological activity, they can effectively used as the therapeutics for a number of diseases, such as, for example the disease in which cells are abnormally died, dementia, cerebral stroke, AIDS, diabetes, gastric ulcer, hepatic injure by hepatitis, sepsis, organ transplantation rejection reaction and anti-inflammation.

The compounds of the present invention therefore may be used as medicines against above-mentioned diseases. Said use as a medicine or method of treatment comprises local or systemic administration to patients of an effective amount of the compounds according to the invention for treating the diseases.

The subject compounds may be formulated into various pharmaceutical forms for administration purposes. Said pharmaceutical forms or compositions are deemed novel and consequently constitute a further aspect of the present invention. Also the preparation of said composition constitutes a further aspect of the present invention. To prepare the pharmaceutical composition of this invention, an effective amount of the compound, in base or salt form, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical composition are desirably in unitary dosage form suitable, preferably, for administration orally, percutaneously, or by parenteral injection.

For example, in preparing the composition in oral dosage form, any of the

usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions: or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agent and the like in the case of powders, pills, capsules and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example to aid solubility, may be included. Injectable solutions, for example may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. In the composition suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agents and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on or as an ointment.

It is especially advantageous to formulate the above pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit as used in the specification and claims herein refers to physically discrete units suitable as unitary dosage, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required

pharmaceutical carrier. Examples of such dosage unit forms are tablets, capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

In view of the usefulness of the subject compounds in the treatment of the disease in which cells are abnormally died, dementia, cerebral stroke, AIDS, diabetes, gastric ulcer, hepatic injure by hepatitis, sepsis, organ transplantation rejection reaction and anti-inflammation, it is evident that the present invention provides a method of treating the subject suffering from the diseases, said method comprising the local or systemic administration of a pharmaceutically effective amount of the compound of formula (I) or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutical carrier. Those skilled in the treatment of the diseases associated could easily determine the effect amount. In general it is contemplated that an effective amount would be from 0.01 mg/kg to 4 mg/kg body weight. However, it is evident to those skilled in the art that such amount ranges are guidelines only and are not intended to limit the scope or use of the invention to any extent.

EXAMPLES

The present invention will be described in greater detail through the following examples. The examples are presented for illustrating purposes only and should not be construed as limiting the invention which is properly delineated in the claims.

(A) Hydroxamoyl chloride synthesis (Examples 1 to 4)

Example 1: Synthesis of N-t-butoxycarbonyl-(S)-valinal and

N-t-butoxy-carbonyl- (S)-valinal oxime

To a solution of dimethyl sulfoxide (11.7 mL, 3.0 eq) in dry CH_2Cl_2 (~200 mL) under N_2 at -60 °C was added slowly oxalyl chloride (5.78 mL, 1.2 eq). After 10 min., a solution of N-t-butoxycarbonyl-(S)-valinol (11.23g, 55.2 mmol) in CH_2Cl_2 (30 mL) was added slowly, and the flask was rinsed with 20 mL of CH_2Cl_2. The resulting white suspension was stirred for 1h at ~ -50 °C. The reaction solution was treated with diisopropylethylamine (28.8 mL, 3.0 eq) and stirred for about 20 min. at -23 °C then diluted with hexanes (400 mL). The mixture was washed with water(150 mL), 1N-$KHSO_4$ solution (x3, total 1 L), dried with anhydrous Na_2SO_4, filtered and concentrated. The yellowish liquid obtained was used directly in next step without further purification.

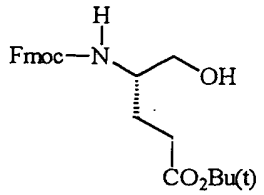
The crude valinal in ethanol (60 mL)-water (30 mL) at water bath temperature was treated with hydroxylamine hydrochloride (5.76g, 1.5 eq) and Na_2CO_3 (4.39g, 0.75 eq.). The reaction generated a lot of solid in 1 min., thus diluted with ethanol-water (1:1, 60 mL) and stirred for 1h. The reaction solution was poured into saturated NaCl (100 mL), then extracted with ethyl acetate twice (300 mL). Organic extracts were washed with sat'd $NaHCO_3$ (100mL x 2), dried (anh. Na_2SO_4), filtered and concentrated to yield white powder (11.34g , syn,anti mixture of oximes).

Example 2: Synthesis of (2S)-2-(t-butoxycarbonyl)amino-1-chloro-3-methyl-butane-1-one oxime

N-t-butoxy-carbonyl-(S)-valinal oxime (11.34g) in DMF (100 mL) was treated with NCS (7.75g) and stirred in warm water bath (~40 °C) for 1h. After removal of DMF, the residue was extracted with ethyl

acetate-hexanes (1:1, 150 mL), washed with water (100 mL x 3), dried (anh. Na_2SO_4), filtered and concentrated to give 13.69g of the title compound.

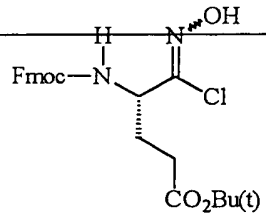
Example 3: Synthesis of 4-(9-fluorenylmethoxycarbonyl)amino-(4S)-5-hydroxy-pentanoic acid t-butyl ester



To a solution of N-(9-fluorenylmethoxycarbonyl)-γ-t-butyl glutamic acid (8.51g, 20.0 mmol) and NMM (2.42mL, 1.1 eq) in dry THF (110 mL) under N_2 at 0 °C was added isobutyl chloroformate (2.72mL, 1.05eq). After 20 min., the reaction mixture was filter-added to a solution of $NaBH_4$ (1.5g) in THF (120mL)-MeOH (30 mL) at -78 °C under N_2 and rinsed with dry THF (20mL). After stirring for 2.5h at -78 °C, the reaction was quenched with acetic acid (13mL). After concentrating to ~ 50mL, the residue was dissolved in ethyl acetate-hexanes (200 mL,1:1), washed with water (150 mL x 2). Aqueous layer was reextracted with ethyl acetate-hexanes (150 mL,1:1). Combined extract was washed with sat'd $NaHCO_3$ (150 mL x 2), dried (anhydrous Na_2SO_4), filtered and concentrated to give 8.30g of the title compound as glasslike solid. The crude alcohol was used directly.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J=7.3Hz), 7.66 (2H, d, J = 7.8 Hz), 7.41 (2H, t, J = 7.3 Hz), 7.31 (2H, pseudo t, J = 7.8, 7.3 Hz), 5.18 (NH, d), 4.41 (2H, m), 4.22 (1H, m), 3.72-3.57 (3H, m), 2.33 (2H, m), 1.93-1.77 (2H, m), 1.45(9H, s).

Example 4: Synthesis of 4-(9-fluorenylmethyloxycarbonyl)amino-(4S)-5-chloro-5- hydroxyimino-pentanoic acid t-butyl ester



To a solution of DMSO (3.0 mL) in dry CH_2Cl_2 (100 mL) at -65°C under N_2 was added oxalyl chloride (2.10 mL, 1.2eq) slowly. After 15 min., a solution of 4-(9-fluorenylmethyloxycarbonyl)amino-(4S)-5-hydroxypentanoic acid t-butyl ester (8.30 g, 20 mmol) in CH_2Cl_2 (50 mL) was added and rinsed with dry CH_2Cl_2 (20 mL). The resulting solution was stirred for 2h at -40 ~ -50 °C. $EtN(i\text{-}Pr)_2$ (10.45 mL, 3.0eq) was added thereto and the reaction solution was slowly warmed up to -10 °C with TLC checking (conversion to aldehyde is relatively slow, ~1h). The reaction mixture was diluted with hexanes (300 mL), washed with water(150 mL), with 1N-$KHSO_4$ (x 3, total 500 mL), dried with anh. Na_2SO_4, filtered and concentrated to give corresponding aldehyde.

The crude aldehyde in ethanol(60 mL)-CH_2Cl_2 (30 mL)-water(10 mL) at 0°C was treated with $H_2NOH \cdot HCl$ (2.08 g, 1.5eq) and Na_2CO_3 (1.60g, 0.75 eq). The reaction was stirred at room temperature for 30 min., then water (10 mL) was added and stirred for additional 1h. The reaction was stirred further(1h) with additional $H_2NOH \cdot HCl$ (400 mg) and Na_2CO_3 (320 mg). Most of the volatiles were removed in vacuo, and the residue was taken up with ethyl acetate (200 mL), washed with water(100 mL), sat'd $NaHCO_3$ (100 mL), dried (anh. Na_2SO_4), filtered and concentrated to give the desired oxime (8.30g, syn + anti) as white powder.

The crude oxime in DMF (35 mL) was treated with NCS (2.67g, 20.0 mmol). The reaction was stirred in warm (40°C) bath for 1h. After removal of the DMF in high vacuum rotary evaporator, the residue was taken up with hexane-ethyl acetate (1:1, 150 mL), washed with water (100 mL x 3), dried (anh. Na_2SO_4), filtered and concentrated to give the title compound (9.25g, syn + anti).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.88(1H, s), 7.75(2H, d, J = 7.3Hz), 7.57(2H, m), 7.39(2H, t, J = 7.32Hz), 7.30 (2h, pseudo t, J = 7.8,7.3Hz), 5.46(1H, d), J = 9.3 Hz), 4.63(1H, m), 4.43-4.38(2H, m), 4.19(1H, m), 2.3(2H, m), 2.03(2H, m), 1.43(9H, s). (NMR data reported for major isomer.)

Following compounds were similarly prepared.

· 1-chloro-3-methyl-(2S)-2-phenylmethyloxycarbonylamino-butane-1-one oxime,

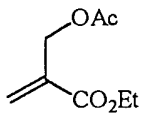
· 3-(t-butoxycarbonylamino)-(3S)-4- chloro-4-hydroxyimino-butanoic acid methyl ester,

· 3-(phenylmethyloxycarbonylamino)-(3S)-4-chloro-4-hydroxyimino-butanoic acid t-butyl ester, and

· 3-(9-fluorenylmethyloxycarbonylamino)-(3S)-4-chloro-4-hydroxyimino-butanoic acid t-butyl ester.

(B) Synthesis of acrylate derivatives (Examples 5 to 8)

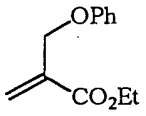
Example 5: Synthesis of ethyl 2-acetoxymethylacrylate



A solution of ethyl 2-hydroxymethyl acrylate (17.3g, 133 mmol, purity ~ 70%, ref: Villieras, J. and Rambaud, M. Synthesis, **1982**, *914*) in dry CH_2Cl_2 (200 mL) under N_2 at 0 °C was treated with acetic anhydride (18.8 mL, 1.5 eq) and triethyl amine (37 mL, 2.0 eq). After overnight stirring at room temperature, the reaction was diluted with hexanes (400 mL), washed with sat'd $NaHCO_3$ (300 mL x 2), dried (anh Na_2SO_4), filtered and concentrated. Simple distillation gave 4.6g of the title compound as clear liquid. NMR analysis showed ~ 70 % purity.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.36 (1H, s), 5.84 (1H, s), 4.81(2H, s), 4.25 (2H, q, J = 7.3 Hz), 2.11 (3H, s), 1.31 (3H, t, J = 7.3 Hz)

Example 6: Synthesis of ethyl 2-phenoxymethylacrylate



A solution of ethyl 2-bromomethylacrylate (2.00g, 10.4 mmol, ref: Villieras, J. and Rambaud, M. Synthesis, **1982**, *914*) and phenol(975 mg, 1.0eq) in dry THF (20 mL) under N_2 at 0 °C was treated with anhydrous K_2CO_3 (1.43g, 1.0 mol eq). No reaction was observed for 1h. Anhydrous DMF (20 mL) was added and stirred for 2h at 0 °C and for 1h at room temperature. After evaporation of DMF, water(100 mL) was added, and the reaction was extracted with ethyl acetate (100 mL x 2). The organic extract was washed with brine (100 mL), dried (anh. Na_2SO_4), filtered and concentrated. Flash chromatography (40% CH_2Cl_2/hexanes) gave 1.712g

(80%) of the title compound.

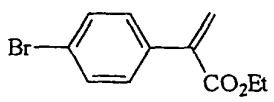
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.30 (2H, yt, J = 7.3 Hz), 6.99-6.96 (3H, m), 6.41 (1H, s), 6.01 (1H, s), 4.78 (2H, s), 4.27 (2H, q, J = 7.33 Hz)

Example 7: Synthesis of ethyl 2-benzylacrylate

To a solution of bromobenzene (7.15g, 45.5 mmol) in THF (30mL) was added n-BuLi (16.6mL, 2.5M in Hexane, 41.4mmol) under N_2 at -78 °C. It was stirred for 10min. To a suspension of CuCN (3.71g, 41.4mmol) in THF (30mL) was added lithiated benzene solution via cannula under N_2 at -78 °C. The reaction mixture was stirred for another 10 min. at -78 °C and ethyl 2-bromomethyl acrylate (4.00g, 20.7 mmol) in THF was added. The reaction mixture was warmed up to room temperature slowly and quenched with 2N HCl. All precipitates were filtered off and filtrate was diluted with hexanes (400 mL), washed with sat'd $NaHCO_3$ (300 mL x 2), dried (anh Na_2SO_4), filtered and concentrated. Flash chromatography (2% ethyl acetate-hexanes) gave 3.04g(77%) of the title compounds .

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.34-7.22 (5H, m), 6.26 (1H, s), 5.48(1H, s), 4.22(2H, q, J = 6.3Hz), 3.66 (2H, s), 1.29 (3H, q, J = 6.3 Hz).

Example 8: Synthesis of ethyl 2-(4-bromophenyl)acrylate

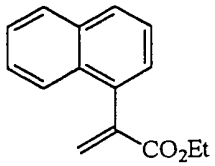


The title compound was prepared following the known procedure (Helvetica Chimica Acta **1986**, *69* 2048).

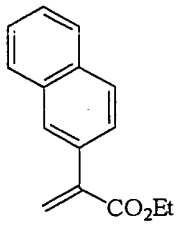
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.46 (2H, d), 7.29 (2H, d), 6.37 (1H, s), 5.90 (1H, s), 4.29 (2H, q), 1.33 (3H, t)

Following compounds were similarly prepared.

. Ethyl 2-(1-naphthyl)acrylate



^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.86 (2H, t, J = 7.3 Hz), 7.44 (1H, d, J = 8.8 Hz), 7.48-7.43 (3H, m), 7.37 (1H, d, J = 6.8 Hz), 6.70 (1H, d, J = 2.0 Hz), 5.89 (1H, d, J = 2.0 Hz), 4.22 (2H, q, J = 7.3 Hz), 1.21 (3H, t, J = 7.3 Hz),

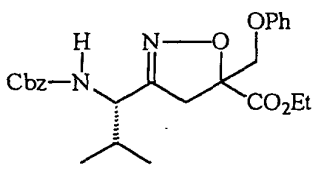


. Ethyl 2-(2-naphthyl)acrylate

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (1H, s), 7.90-7.86 (3H, m), 7.59-7.52 (3H, m), 6.47 (1H, d, J = 1.0Hz), 6.06 (1H, d, J = 1.0 Hz), 4.38 (2H, q, J = 6.8 Hz), 1.40 (3H, t, J = 6.8Hz).

(C) General procedure for isoxazoline synthesis (Examples 9 and 10)

Example 9: Synthesis of 3-((1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl)-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester



A solution of (2S)-2-phenylmethyloxycarbonylamino-1-chloro-3-methyl-butane-1-one oxime (640 mg, 2.25mmol) and ethyl 2-phenoxymethylacrylate (464mg) in dry ether(10 mL) under N_2 at -78°C was treated with triethylamine (627 uL, 2.0 eq). The reaction was stirred overnight, allowing to warm up to room temperature slowly. Water(100 mL) was added, and the reaction was extracted with ethyl acetate (100 mL x 2), washed with water(100mL), dried (anh. Na_2SO_4), filterd and concentrated. Flash chromatography (15% ethyl acetate-hexanes) gave 851mg(83%) of the title compounds as 1:1 mixture of diastereomers.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.34(7H, m), 6.98 (1H, t, J = 7.3Hz), 6.89 (2H, d, J = 7.7Hz), 5.61 (1H, d, J = 9.3 Hz), 5.15-5.08 (2H, m), 4.50 (1H, br s), 4.33-4.22 (4H, m), 3.60-3.54(1H, m), 3.32-3.27(1H, m), 2.10 (1H, m), 1.29 (3H, m), 1.02-0.94 (6H, m).

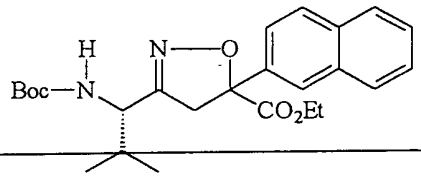
The following compounds were prepared similarly:

· Ethyl 3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carboxylate (diastereomeric)

1H-NMR (500 MHz, $CDCl_3$) δ 7.45-7.15 (m, 10H), 5.07 (m, 2.5H), 4.90 (d, 0.5H), 4.30-4.18 (m, 3H), 3.36-2.88 (m, 4H), 1.95-1.80 (m, 1H), 1.27 (m, 3H), 0.86-0.55 (m, 6H).

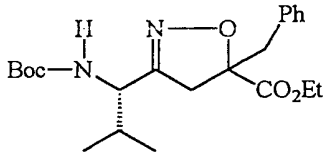
· 3-[(1S)-1-t-butoxycarbonylamino-2-methyl-propyl]-5-(2-naphthyl)-4,5-di-

hydro-isoxazole-5-carboxylic acid ethyl ester



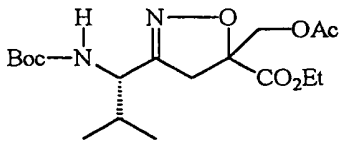
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.97(1H, s), 7.86-7.82 (3H, m), 7.52-7.48 (3H, m), 4.93 (1H, br), 4.37 (1H, m), 4.25 -4.18 (2H, m), 4.10-4.05 (1H, two doublets, J=17.1, 17.6 Hz), 3.28-3.22 (1H, two doublets, J = 17.1, 17.1 Hz), 2.05 (1H, m), 1.43 ((H, s), 1.24-1.20 (3H, m), 0.98-0.91 (6H, m).

· 3-[(1S)-1-t-butoxycarbonylamino-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro- i-soxazole-5-carboxylic acid ethyl ester (~1:1 diastereomers)



^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.25 (5H, m), 4.82 and 4.60 (1H, two m), 4.25-4.15 (3H, m), 3.38-3.29 (2H, m), 3.10 (1H, m), 2.90 (1H, m), 1.43 and 1.42 (9H, two s), 1.27 (3H, m), 0.90-0.80 (6H, m).

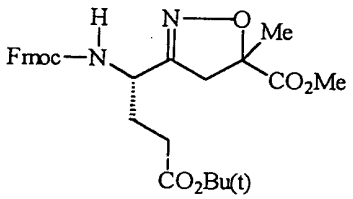
· 5-acetoxymethyl-3-[(1S)-1-t-butoxy-carbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.93 (1H, br), 4.44-4.26 (5H, m), 3.50

(1H, m), 3.10 (1H, m), 2.08 (4H, s + br 1H), 1.46 (9H, s), 1.32-1.30 (3H, m), 1.02-0.96 (6H, m).

Example 10: Synthesis of 3-[(1S)-1- (9-fluorenylmethyloxycarbonylamino)-3-t-butoxycarbonyl-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester



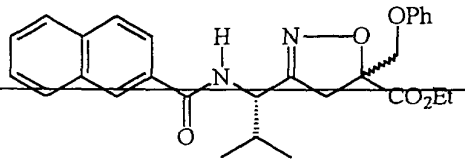
A solution of 4-(9-fluorenylmethoxycarbonyl)amino-(4S)-5-chloro-5-hydroxy-imino-pentanoic acid t-butyl ester (3.44g, 7.50 mmol) and methyl methacrylate (2.40mL, 3.0 eq) in dry ether under N_2 at -78 °C was treated with $EtN(i\text{-}Pr)_2$ (1.96mL, 1.5eq). Similar treatment as described previously followed by flash chromatography with 25-30% ethyl acetate/hexanes gave 3.46g (89% overall) of the title compound as diastereomeric mixture.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J=7.3Hz), 7.59 (2H, d, J =7.3Hz), 7.40 (2H, t, J = 7.3Hz), 7.31 (2H, t, J =7.3 Hz), 5.34 (1H, m), 4.58-4.38 (3H, m), 4.21 (1H, m), 3.78 (3H, s), 3.48 (1H, m), 2.90-2.81 (1H, m), 2.42-2.27 (2H, m), 2.18 (1H, m), 1.93 (1H, m), 1.63 (3H, s), 1.45 (9H, s)

(D) Transformations of isoxazolines (Deprotection, Introduction of P_4 group, Hydrolysis of ester group) (Examples 11 and 12)

Example 11: Synthesis of 3-{2-methyl- (1S)-1-(naphthalene-2-carbonyl-

amino)-propyl}-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester



A solution of 3-{(1S)-1-(t-butoxycarbonylamino)-2-methyl-propyl}-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester (2.00g, 4.76 mmol) in dry CH_2Cl_2 (10 mL) at 0°C under N_2 was treated with TFA (6 mL) and stirred for 1.5h. After removal of volatiles, the residue was taken up with ethyl acetate (200 mL), washed with sat'd $NaHCO_3$ (100 mL x 2), dried (anh Na_2SO_4), filtered and concentrated. To a solution of the crude product, EDC (1.09g, 1.2 eq), 2-naphthoic acid (983 mg, 1.2 eq) and HOBt (771 mg, 1.2 eq) in DMF (20 mL) at 0°C was added triethylamine (663 uL, 1.0 eq). The reaction was stirred overnight at room temperature. After removal of volatiles in vacuo, the residue was taken up with ethyl acetate (250 mL), washed with water(100 mL), sat'd $NaHCO_3$ (100 mL x 2), dried (anh Na_2SO_4), filtered and concentrated. Flash chromatography with 25-33% ethyl acetate/hexanes gave 2.04g (90%) of the title compound.

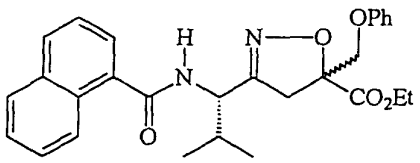
^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (1H,s), 7.93-7.84 (4H, m), 7.58-7.52 (2H, m), 7.29-7.22 (2H, m), 7.00-6.81 (4H, m), 5.06-5.01 (1H, m), 4.36-4.24 (4H, m), 3.68-3.61 (1H, m), 3.43-3.39 (1H, m), 2.28 (1H, m), 1.31-1.26 (3H, m), 1.12-1.05 (6H, m).

Hydrolysis of isoxazoline 5-carboxylic acid ester: The above compound (2.04g) in distilled THF (40 mL) (not completely soluble) was treated with 1N-NaOH(5.2 mL, 1.2 eq). After 4h (~50% completion), additional

1N-NaOH (1.0 mL) was added. After overnight stirring, the reaction was neutralized with concentrated 1N-HCl. The residue was taken up with CH_2Cl_2 (>700 mL), washed with water, dried (anh Na_2SO_4), filtered and concentrated to give 1.948g (103%) of the free carboxylic acid, which was used directly in next step.

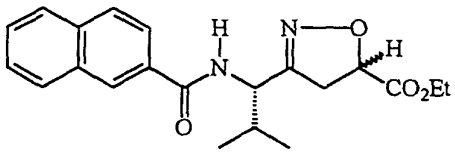
The following compounds were prepared similarly:

· 3-{2-methyl-(1S)-1-(naphthalene-1-carbonylamino)-propyl}-5-phenoxymethyl -4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.23 (1H, d, J = 8.3 Hz), 7.93-7.86 (2H, m), 7.66 (1H, m), 7.54-7.42 (3H, m), 7.29-7.25 (2H, m), 7.00-6.90 (3H, m), 6.49 (1H, m), 5.13-5.09 (1H, m), 4.40-4.26 (4H, m), 3.69-3.64 (1H, m), 3.44-3.41 (1H, m), 2.28 (1H, m), 1.32-1.01 (9H, m).

· 3-{2-methyl-(1S)-1-(naphthalene-2-carbonylamino)-propyl}-4,5-dihydro-isoxa zole-5-carboxylic acid ethyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, s), 7.94-7.83 (4H, m), 7.59-7.53 (2H, m), 6.80-6.70 (NH, two d), 5.07-5.03 (2H, m), 4.28-4.21 (2H, m), 3.37-3.33 (2H, m), 2.28 (1H, m), 1.34-1.25 (3H, m), 1.12-1.02 (6H, m).

. 3-[(1S)-1-(1-naphthalenecarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4, 5- dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 7.8 Hz, 1H), 7.94-7.86 (m, 2H), 7.61-7.11 (m, 9H), 6.36 (d, J = 9.3 Hz, 0.5H), 6.09 9d, J = 9.3 Hz, 0.5H), 4.94-4.85 (m, 1H), 4.27-4.21 (m, 2H), 3.49-2.98 (m, 4H), 2.15 & 1.97 (two m, 1H), 1.30-1.26 (m, 3H), 1.03-0.59 (m, 6H).

. Ethyl 3-[(1S)-1-phenethylcarbonylamino-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carboxylate (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.28-7.17 (m, 10H), 5.74 & 5.50 (two d, J = 9.3 Hz, NH), 4.58-4.52 (m, 1H), 4.24-4.20 (m, 2H), 3.34-3.25 (m, 2H), 3.11-2.82 (m, 4H), 2.52-2.45 (m, 2H), 1.93 &1.75 (two m, 1H), 1.29-1.25 (m, 3H), 0.79-0.41 (m, 6H).

. 3-[(1S)-1-(1-naphthalenesulfonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5 -dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.68-8.64 (m, 1H), 8.29-8.25 (m, 1H), 8.07 (m, 1H), 7.93 (m, 1H), 7.71-7.52 (m, 3H), 7.23-6.98 (m, 5H), 5.27 & 5.19 (two m, 1H), 4.12-4.07 (m, 2H), 3.75 & 3.66 (two m, 1H), 3.16-2.43 (m, 4H), 1.77-1.62 (m, 1H), 1.25-1.16 (m, 3H), 0.86-0.57 (m, 6H).

. 3-[(1S)-1-(indole-3-yl-ethylcarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5 -dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.16-8.12 (m, 1H), 7.62-7.56 (m, 1H), 7.36-6.94 (m, 9H), 5.71 (d, J = 9.3 Hz, 0.5H), 5.42 (d, J = 8.8 Hz, 0.5H), 4.56-4.50 (m, 1H), 4.25-4.17 (m, 2H), 3.30-2.51 (m, 8H), 1.89-1.70 (m, 1H), 1.28-1.24 (m, 3H), 0.73-0.41 (m, 6H).

. 3-[(1S)-1-(indole-3-yl-methylcarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5 -dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.56 & 8.52 (two br s, 1H), 7.55-7.05 (m, 10H), 5.98-5.91 (m, 1H), 4.57 (m, 1H), 4.22-4.15 (m, 2H), 3.73 (m, 2H), 3.28-2.79 (m, 4H), 1.87-1.68 (m, 1H), 1.27-1.20 (m, 3H), 0.75-0.34 (m, 6H).

. 3-[(1S)-1-(cinnamoylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

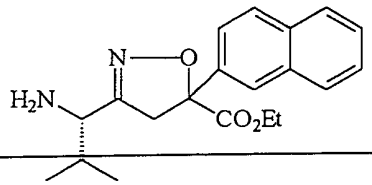
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.61-7.23 (m, 11H), 6.40-6.34 (m, 1H), 6.06 (d, J = 8.8 Hz, 0.5H), 5.81 (d, J = 9.3 Hz, 0.5H), 4.76-4.69 (m, 1H), 4.26-4.19 (m, 2H), 3.42-2.94 (m, 4H), 2.06 & 1.88 (two m, 1H), 1.28-1.24 (m, 3H), 0.93-0.57 (m, 6H).

. 3-[(1S)-1-(phenylmethylsufonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.35-7.16 (m, 10H), 4.66-4.61 (m, 1H), 4.25 (m, 2H), 4.11-3.84 (m, 3H), 3.71-2.82 (m, 4H), 1.80 & 1.70 (two m, 1H), 1.28 (m, 3H), 0.85-0.58 (m, 6H).

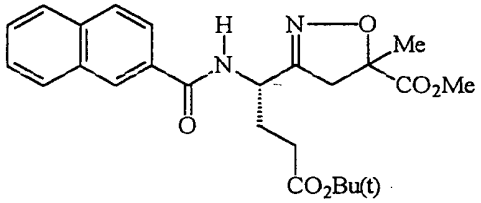
. 3-[2-methyl-(1S)-1-amino-propyl]-5-(2-naphthyl)-4,5-dihydro-isoxazole-5-car

boxylic acid ethyl ester (~1.3:1 diastereomers)



^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.99 (1H, s), 7.86-7.82 (3H, m), 7.53-7.49 (3H, m), 4.25-4.02 (3H, m), 3.55-3.48 (1H, two d, J = 7.3, 6.8Hz), 3.35 (0.45H, d, J=17.1 Hz), 3.19 (0.55H, d, J = 17.1Hz), 1.78 (1H, m), 1.22 (3H, t, J = 7.3 Hz), 0.96-0.82 (6H, m)

Example 12: Synthesis of 3-{(1S)-1- (2-naphthoylamino)-3-t-butoxycarbonyl-propyl}-5-methyl-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester



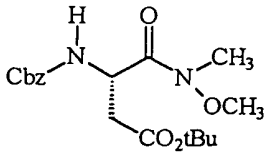
A solution of 3-[(1S)-1-(9-fluorenylmethyloxycarbonylamino)-3-t-butoxy-carbonyl-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester (440mg, 0.842 mmol) in DMF (8.0 mL) at room temperature was treated with piperidine (2.5 mL) for 5 min. After concentration, the residue was dissolved in DMF (10 mL), and treated with 2-naphthoic acid (174 mg, 1.2 eq), EDC (210 mg, 1.3 eq), HOBt (148 mg, 1.3 eq) and triethylamine (0.35 mL, 3.0 eq), then stirred overnight (0°C toroom temperature). Usual workup followed by chromatography gave 133 mg of the title compound and 260 mg (~50% purity) mixture.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.33 (1H, s), 7.92-7.83 (4H, m), 7.58-7.48

(2H, m), 7.34 (1H, d, J=7.8Hz), 5.04 (1H, m), 3.78 and 3.74 (3H, two s), 3.62-3.53 (1H, two d, J=17.1, 17.6Hz), 3.00-2.96 (1H, two d, J =17.1, 17.6 Hz), 2.56-2.08 (4H, m), 1.63 and 1,59 (3H, two s), 1.41 and 1.40 (9H, two s)

(E) Synthesis of aspartic acid derivatives (Examples 13 to 18)

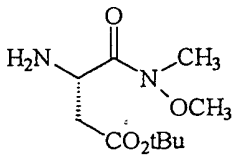
Example 13: Synthesis of N-phenylmethyloxycarbonyl-β-t-butyl aspartic acid (N-methoxy) methyl amide



A solution of N-benzyloxycarbonyl-β-t-butyl aspartic acid (2.0g, 6.2 mmol), N,O-dimethylhydroxylamine hydrochloride (724 mg, 1.2 eq) and HOBt (1.00g, 1.2 eq) in DMF (20 mL) at 0°C was treated with EDC (1.42g, 1.2 eq) and triethylamine (1.29 mL, 1.5 eq). After overnight stirring (0°C toroom temperature), the reaction was diluted with water(100mL), extracted with ethyl acetate-hexanes (1:1, 100 mL x 2), washed with water(100 mL), dried (anh Na_2SO_4), filtered and concentrated. Flash chromatography with ethyl acetate-hexanes (3:7) gave 2.039g (90%) of the title compound.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36-7.31 (5H, m), 5.70 (1H, br), 5.16-5.08 (3H, m), 3.80 (3H, s), 3.23 (3H, s), 2.74-2.71(1H, m), 2.59 -2.57 (1H, m), 1.43 (9H, s).

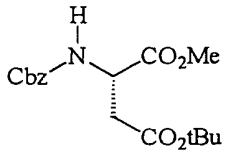
Example 14: Synthesis of β-t-butyl aspartic acid N,O-dimethylhydroxyl-amine amide



Conventional hydrogenolysis of N-phenylmethyloxycarbonyl-β-t-butyl aspartic acid (N-methoxy)methyl amide (H_2 balloon, 10% Pd/C, EtOH) gave the title compound (100%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.13 (1H, m), 3.77 (3H, s), 3.22 (3H, s), 2.71-2.67 (1H, m), 2.42-2.38 (1H, m), 1.46 (9H, s)

Example 15: Synthesis of N-phenylmethyloxycarbonyl-β-t-butyl aspartic acid methyl ester



Treatment of N-benzyloxycarbonyl-β-t-butyl aspartic acid with diazomethane/ ether gave the desired methyl ester (100%).

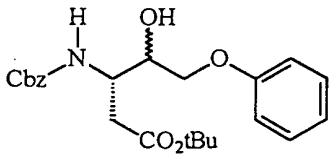
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.35-7.27 (5H, m), 5.75 (1H, d), 5.13 (2H, s), 4.60 (1H, m), 3.75 (3H, m), 2.90 (1H, m), 2.76 (1H, m), 1.42 (9H, s).

Example 16: Synthesis of β-t-butyl aspartic acid methyl ester hydrochloride

Conventional hydrogenolysis of N-phenylmethyloxycarbonyl-β-t-butyl

aspartic acid methyl ester (H_2 balloon, 10% Pd/C, EtOH-HCl) gave the desired product as hydrochloride salt.

Example 17: Synthesis of (3S)-3-phenylmethyloxycarbonylamino-4-hydroxy-5-phenoxy-pentanoic acid t-butyl ester



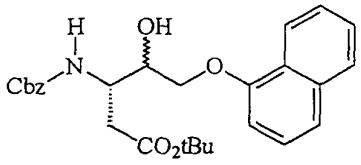
A solution of N-phenylmethyloxycarbonyl-β-t-butyl-aspartic acid (5.03g, 15.6 mmol), NMM (1.90 mL, 17.1 mmol) in dry THF (60 mL) under N_2 at -15°C was treated with isobutyl chloroformate (2.12 mL, 16.3 mmol) and the resulting suspension was stirred for 20 min. To the mixture at 0°C was added dry diazomethane/ether (synthesized from 2.0 eq of 1-methyl-3-nitro-1- nitroso-guanidine, 60 mL) and stirred for 30 min. When the diazo ketone synthesis was completed (TLC analysis), 30% HBr/AcOH (6.42 mL, 2.0 eq) was introduced thereto (stirred for 30-60 min.) at 0°C. The reaction was extracted with ethyl acetate, washed with sat'd $NaHCO_3$ (x 2), brine, dried (anh. Na_2SO_4), filtered and concentrated to give bromomethyl ketone derivative (6.4g).

The bromomethyl ketone(4.36g) and phenol (1.13g, 1.1 eq) in DMF (18 mL) at room temperature was treated with freshly dried KF (1.58g, 2.5 eq) and stirred for 2 h. Usual extractive workup gave crude phenoxy ketone. The crude phenoxy ketone in methanol (20 mL) at -78 °C was treated with $NaBH_4$ (412 mg) in MeOH (40 mL) (78 °C toroom temperature, 2h). The reaction was quenched with acetic acid. Usual extractive workup followed by flash chromatography (ethyl acetate-hexanes = 1:5) gave 2.58g (57%) of the titie compound as diastereomeric mixture.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36-7.26 (7H, m), 6.98-6.87 (3H, m), 5.71-5.53 (NH, two d), 5.10 (2H, s), 4.24-3.92 (4H, m), 2.70-2.63 (2H, m), 1.44 and 1.43 (9H, two s).

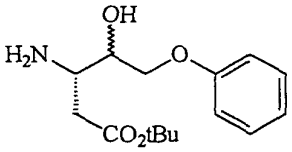
Following compound was prepared similarly:

. (3S)-3-phenylmethyloxycarbonylamino-4-hydroxy-5-(1-naphthyl)oxy-pentanoic acid t-butyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.21 (1H, m), 7.80 (1H, m), 7.50-7.33 (9H, m), 6.80 (1H, m), 5.73 and 5.55 (1H, two d, J = 8.3 Hz), 5.10 (2H, s), 4.30-4.15 (4H, m), 2.76-2.69 (2H, m), 1.44 (9H, s).

Example 18: Synthesis of (3S)-3-amino-4-hydroxy-5-phenoxy-pentanoic acid t-butyl ester



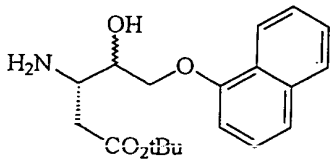
Conventional hydrogenolysis of (3S)-3-phenylmethyloxycarbonylamino-4-hydroxy-5-phenoxy-pentanoic acid t-butyl ester (H_2 balloon, Pd/C, EtOH) gave the desired product (100%).

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.29-7.26 (2H, m), 6.97-6.90 (3H, m),

4.08-3.82 (3H, m), 3.43 (1H, m), 2.63-2.37 (2H+NH_2+OH, m), 1.46 and 1.45 (9H, two s).

The following compound was prepared similarly:

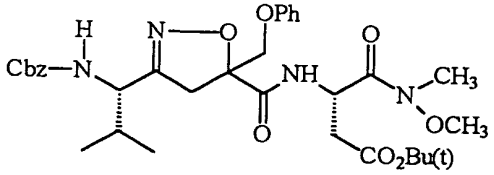
· (3S)-3-amino-4-hydroxy-5-(1-naphthyl)oxy-pentanoic acid t-butyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.22 (1H, m), 7.80 (1H, m), 7.50-7.34 (4H, m), 6.84 (1H, m), 4.26-4.20 (2H, m), 4.03-3.94 (1H, m), 3.51 (1H, m), 2.70-2.40 (2H, m), 1.47 and 1.46 (9H, two s).

(F) Coupling of isoxazoline derivatives and aspartic acid derivatives and further transformations thereof (Examples 19 to 24).

Example 19: Synthesis of (2S)-2-{3-[(1S)-1-phenylmethyloxycarbonyl-amino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-(N-methyl-N-methoxy) amide



A solution of 3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester (502mg, 1.10 mmol) in THF (6.6 mL) was treated with 1N-NaOH (1.33mL). After stirring for 2.5h at room temperature, the reaction was quenched with

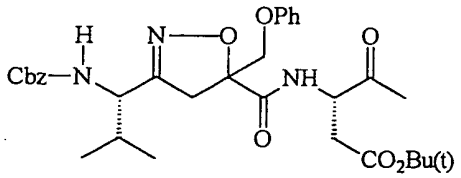
1N-HCl (1.33 mL), then concentrated in vacuo. The residue together with sat'd NaCl(50 mL+ 2-3 mL의 1N-HCl) was extracted with ethyl acetate (100 mL x 2), dried (anh Na_2SO_4), filtered and concentrated to give 476mg (101 %) of 3-[(1S)-1-phenylmethyl-oxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carboxylic acid.

The crude acid (320 mg, 0.75 mmol) and β-t-butyl aspartic acid N-methyl- (N-methoxy) amide (209 mg, 1.2 eq) in DMF (5mL) at 0°C was treated with HOBt (122mg, 1.2 eq), EDC (172mg, 1.2 eq) and triethylamine (0.31 mL, 3.0 eq) and stirred for 3h (0°C to room temperature). Concentration, conventional workup followed by flash chromatography gave less polar isomer (160mg) and more polar isomer (213mg, 33%).

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.64 (1H, d), 7.35-7.24 (7H, m), 6.95 (1H, t, J = 7.3 Hz), 6.88 (2H, d, J = 7.8 Hz), 5.55 (1H, d), 5.18-5.08 (3H, m), 4.44 (1H, m), 4.32-4.25 (2H, m), 3.75 (3H, s), 3.32-3.25 (2H, m), 3.12 (3H, s), 2.77-2.71(1H, m), 2.62-2.56 (1H, m), 2.12 (1H, m), 1.44 (9H, s), 1.03-0.91 (6H, m).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.65 (1H, d, J = 8.3 Hz), 7.36-7.23 (7H, m), 6.95 (1H, t, J = 7.3 Hz), 6.88 (2H, d, J = 8.3 Hz), 5.19-5.11 (4H, m), 4.46 (1H, m), 4.33-4.22 (2H, ABq, J =10.3 Hz), 3.75 (3H, s), 3.33 (2H, s), 3.23 (3H, s), 2.73 (1H, m), 2.57 (1H, m), 2.07 (1H, m), 1.43 (9H, s), 1.03-0.92 (6H, m).

Example 20: Synthesis of (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonyl-amino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid t-butyl ester

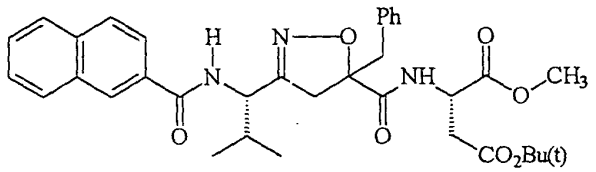


The title compound was obtained from treatment of excess MeMgBr (3.0M in ether, > 3.0 eq) to a solution of less polar isomer of (2S)-2-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxa-zole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-(N-methyl-N-methoxy) amide (110 mg, 0.17 mmol) in THF (5 mL) + LiCl satuated THF (2 mL) at 0°C - room temperature (44mg, 43%).

From less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.00 (1H, d, J = 9.3 Hz), 7.36-7.24 (7H, m), 6.96 (1H, t, J = 7.2 Hz), 6.87 (2H, d, J = 8.3 Hz), 5.26 (1H, d, J = 8.8 Hz), 5.12-5.09 (2H, m), 4.66 (1H, m), 4.43 (1H, d, J= 9.8 Hz), 4.21 (1H, d, J = 9.8 Hz0, 3.37-3.19 (2H, ABq, J = 18.0 Hz), 2.88 (1H, m), 2.58 (1H, m), 2.25 (3H, s), 2.03 (1H, m), 1.42 (9H, s), 0.99-0.89 (6H, m).

Similar treatment of more polar isomer of (2S)-2-{3-[(1S)-1-phenylmethyl-oxy-carbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-(N-methyl-N-methoxy) amide (135 mg) gave 52mg (41%) of the corresponding methyl ketone.

Example 21: Synthesis of (2S)-2-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonyl-amino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-methyl ester



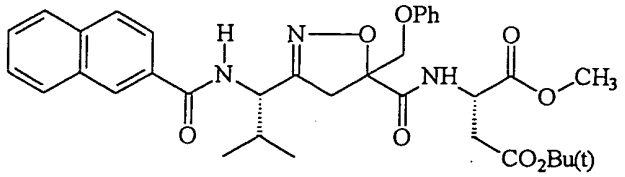
A solution of 3-[2-methyl-(1S)-1-(naphthalene-2-carbonylamino)-propyl]-5-phenyl- methyl-4,5-dihydro-isoxazole-5-carboxylic acid (2.14g, 5.07 mmol), aspartic acid β-t-butyl ester methyl ester hydrochloride (1.46g, 1.2 eq), EDC (1.17g, 1.2 eq) and HOBt (822 mg, 1.2 eq) in DMF (19 mL) was treated with triethylamine (2.12 mL, 3.0 eq), and stirred overnight. Conventional workup followed by flash chromatography (40-50% ethyl acetate-hexanes) gave the title compound (2.94g, 94%)as a white foam.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 and 8.25 (1H, two s), 7.96-7.79 (4H, m), 7.65-7.54 (3H, m), 7.31-7.18 (5H, m), 6.76 (0.5H, d, J = 9.3 Hz), 6.43 (0.5H, d, J = 8.8 Hz), 4.96-4.70 (2H, m), 3.71 and 3.60 (3H, two s), 3.45-3.14 (4H, m), 3.08-2.34 (2H, m), 2.15 (1H, m), 1.47 and 1.44 (9H, two s), 1.04-0.88 (6H, m).

The above compound was hydrolyzed following previously described method (1N-NaOH in THF) to obtain coresponding carboxylic acid (100%).

The following esters and free carboxylic acids were prepared similarly.

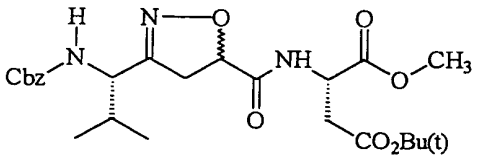
. (2S)-2-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonylamino)-propyl]-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-methyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.33 and 8.30 (1H, two s), 7.95-7.74 (5H, m), 7.59-7.53 (2H, m), 7.28-7.22 (2H, m), 6.99-6.89 (3.5H, m), 6.71 (0.5H, d, J = 8.8 Hz), 5.08-5.01 (1H, m), 4.83-4.79 (1H, m), 4.39-4.29 (2H, m), 3.76 and 3.64 (3H, two s), 3.44 (2H, s), 2.97-2.93 (1H, m), 2.74-2.69 (1H, m), 2.34-2.23 (1H, m), 1.45 and 1.42 (9H, two s), 1.15-1.01 (6H, m).

Hydrolysis of above compound gave free carboxylic acid.

· (2S)-2-{3-[(1S)-1-(phenylmethyloxycarbonyl)-amino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester 1-methyl ester



^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.59-7.49 (1H, m), 7.38-7.32 (5H, m), 5.25-4.95 (4H, m), 4.86 (1H, m), 4.48 (1H, m), 3.76 and 3.67 (3H, two s), 3.29 (2H, m), 2.92 (1H, m), 2.71-2.62 (1H, m), 2.04 (1H, m), 1.48 (9H, s), 1.01-0.85 (6H, m)

· (2S)-2-{3-[(1S)-1-phenethylcarbonylamino-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 8.3 Hz, 0.5H), 7.47 (d, J = 9.3 Hz, 0.5 H), 7.28-7.18 (m, 10H), 5.83 & 5.44 (two d, J = 8.8 Hz, 1H), 4.70-4.52 (m, 2H), 3.68 & 3.65 (two s, 3H), 3.33-2.28 (m, 10H), 1.89 (m, 1H), 1.43 & 1.42 (two s, 9H), 0.79-0.63 (m, 6H).

· (2S)-2-{3-[(1S)-1-(1-naphthalenecarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.27 (m, 1H), 7.92-7.85 (m, 2H), 7.61-7.15 (m, 10H), 6.45 & 6.05 (two d, NH), 4.99-4.85 (m, 1H), 4.70 (m, 1H), 3.69 & 3.52 (two s, 3H), 3.50-2.32 (m, 6H), 2.12 (m, 1H), 1.40 & 1.39 (two s, 9H), 1.05-0.80 (m, 6H).

· (2S)-2-{3-[(1S)-1-(1-naphthalenesulfonylamino-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.69-8.62 (m, 1H), 8.33-7.94 (m, 3H), 7.70-7.47 (m, 3H), 7.20-7.05 (m, 5H), 5.32 & 5.15 (two m, 1H), 4.68 & 4.54 (two m, 1H), 3.85 & 3.59 (two m, 1H), 3.82 & 3.62 (two s, 3H), 3.23-1.75 (m, 7H), 1.40 & 1.34 (two s, 9H), 0.85-0.48 (m, 6H).

· (2S)-2-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.53-7.49 (two d, 1H), 7.35-7.25 (m,

10H), 5.09-5.07 (m, 2.5 H), 4.88 (d, 0.5 H), 4.69 (m, 1H), 4.34 & 4.23 (two m, 1H), 3.68 &3.63 (two s, 3H), 3.36-2.23 (m, 6H), 1.89 & 1.70 (two m, 1H), 1.42 & 1.40 (two s, 9H), 0.88-0.73 (m, 6H).

· (2S)-2-{3-[(1S)-1-(indole-3-yl-ethylcarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.54 & 8.38 (two br s, 1H), 7.62-6.97 (m, 1H), 5.83 (d, J = 8.8 Hz, 0.5H), 5.20 (d, J = 9.3 Hz, 0.5H), 4.73-4.69 (m, 1H), 4.61 & 4.48 (two m, 1H), 3.71 & 3.59 (two s, 3H), 3.28-2.26 (m, 10H), 1.87-1.75 (m, 1H), 1.43 &1.42 (two s, 9H), 0.78-0.50 (m, 6H).

· (2S)-2-{3-[(1S)-1-(indole-3-yl-methylcarbonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl-ester-1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.37 & 8.26 (two br s, 1H), 7.54-7.12 (m, 11H), 5.95 (d, J = 8.8 Hz, 0.5H), 5.76 (d, J = 1.5 Hz, 0.5H), 4.68-4.51 (m, 2H), 3.78-3.68 (m, 2H), 3.66 & 3.62 (two s, 3H), 3.28-2.21 (m, 6H), 1.80 (m, 1H), 1.41 & 1.37 (two s, 9H), 0.75-0.46 (m, 6H).

· (2S)-2-{3-[(1S)-1-(cinnamoylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester-1-methyl ester (diastereomeric)

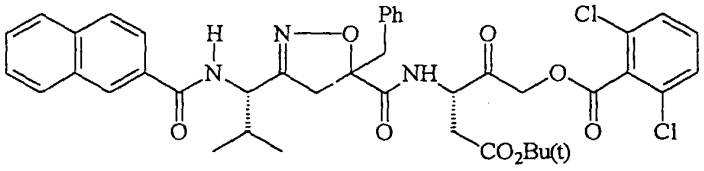
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.63-7.25 (m, 12H), 6.43-6.32 (two d, J = 15.6 Hz, 1H), 6.09 & 5.68 (two d, J = 9.3 Hz, 1H), 4.78-4.70 (m, 1H),

3.69 & 3.68 (two s, 3H), 3.35-2.31 (m, 6H), 2.03 (m, 1H), 1.43 & 1.40 (two s, 9H), 0.92-0.76 (m, 6H).

(2S)-2-{3-[(1S)-1-(phenylmethylsulfonylamino)-2-methyl-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-succinic acid 4-t-butyl ester 1-methyl ester (diastereomeric)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.67 & 7.60 (two d, J = 8.8 Hz, 1H), 7.40-7.17 (m, 10H), 3.71 & 3.55 (two s, 3H), 3.37-2.23 (m, 6H), 1.70 (m, 1H), 1.42 & 1.47 (two s, 9H), 0.91-0.65 (m, 6H).

Example 22: Synthesis of (3S)-3-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonyl-amino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid-t-butyl ester



A solution of (2S)-2-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-4,5-dihydro-5-phenylmethyl-isoxazole-5-carbonyl-amino}-succinic acid 4-t-butyl ester (2.86g, 4.75 mmol) and NMM (0.57 mL, 1.1 eq) in dry THF (x mL) under N_2 at 0°C was treated with isobutyl chlroformate (0.65 mL, 1.05eq), and stirred for 20 min. To the solution at 0°C was added diazomethane, and stirred for 30 min. (TLC analysis). Additional diazomethane was needed to complete the reaction(1h). After completion of the diazoketone formation, 30% HBr/AcOH (4.0 mL, 4.0 eq) was added at 0 oC and the reaction was stirred for 1h. The reaction was extracted with ethyl acetate (x2) and the organic layer was washed with water, sat'd

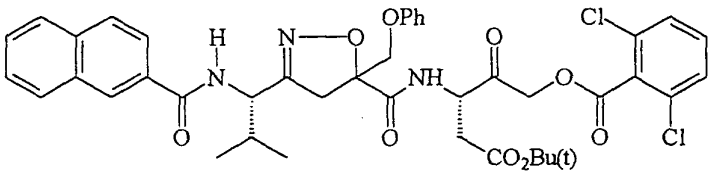
$NaHCO_3$ and brine, dried (anh Na_2SO_4), filtered and concentrated to give 3.36g of yellow solid. Half of the solid (~2.375 mmol) was reacted with anhydrous KF (345 mg, 2.5 eq) and 2,6-dichlorobenzoic acid (545 mg, 1.2 eq) in DMF (10 mL) under N_2 at room temperature. Usual workup followed by flash chromatography gave the title compound as diastereomeric mixture (1.53g). Preparative HPLC (38% EtOAc/Hexane) gave less polar diastereomer (585 mg) and more polar diastereomer (358mg).

Less polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.28 (1H, s), 7.84-7.80 (4H, m), 7.55-7.46 93H, m), 7.29-7.24 98H, m), 6.87 (1H, d, J = 8.8 Hz), 5.05-4.93 (3H, m), 4.73 (1H, m), 3.54 (1H, d, J = 18.1 Hz), 3.34 (1H, d, J = 13.7 Hz), 3.19 (1H, d, J = 14.2 Hz), 3.11 (1H, d, J = 17.6 Hz), 2.74-2.70 (1H, m), 2.29-2.24 (2H, m), 1.39 (9H, s), 1.02 (3H, d, J = 6.4 Hz), 0.92 (3H, d, J = 6.8 Hz).

More polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.28 (1H, s), 7.97-7.75 (5H, m), 7.62-7.57 (2H, m), 7.37-7.22 (8H, m), 6.56 (1H, d, J = 8.3 Hz), 4.94 (1H, m), 4.78 (1H, m), 4.51-4.42 (2H, m), 3.51-3.43 (2H, m), 3.24-3.15 (2H, m), 2.99-2.95 (1H, m), 2.56-2.52 (1H, m), 2.18 (1H, m), 1.45 (9H, s), 1.02 (3H, d, J = 6.8 Hz), 0.97 (3H, d, J = 6.4 Hz).

The following compounds were prepared similarly.

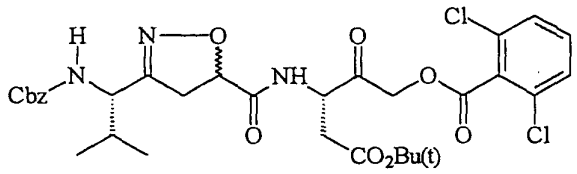
· (3S)-3-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonylamino)-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid-t-butyl ester



Less polar diastereomer : ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s), 7.85-7.81 (5H, m), 7.54-7.46 (2H, m), 7.31-7.23 (5H, m), 6.98-6.87 (4H, m), 5.13-5.03 (3H, m), 4.90 (1H, m), 4.39-4.27 (2H, ABq, J = 9.3 Hz), 3.51 (1H, d, J = 17.6 Hz), 3.41 (1H, d, J = 17.6 Hz), 2.94-2.78 (2H, m), 2.38 (1H, m), 1.41 99H, s), 1.12-1.08 (6H, two d, J = 6.4 Hz).

More polar diastereomer : ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, s), 8.11 (1H, d, J = 8.8 Hz), 7.93-7.83 (4H, m), 7.59-7.53 (2H, m), 7.33-7.22 (5H, m), 6.97-6.91 (3H, m), 6.77 (1H, d, J = 8.8 Hz), 5.37 (1H, d, J = 17.1 Hz), 5.16 (1H, d, J = 17.1 Hz), 5.01-4.95 (2H, m), 4.53 (1H, d, J = 9.8 Hz), 4.25 (1H, d, J = 9.8 Hz), 3.50 (1H, d, J = 7.6 Hz), 3.32 (1H, d, J = 7.6 Hz), 3.04-3.00 (1H, dd, J = 17.1, 4.9 Hz), 2.73-7.68 (1H, dd, 17.1, 5.4 Hz), 2.24 (1H, m), 1.47 (9H, s), 1.10-1.03 (6H, two d, J = 6.4 Hz).

· (3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid-t-butyl ester (diastereomeric mixture)



^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.72-7.60 (1H, m), 7.37-7.30 (8H, m), 5.40 (0.5H, d), 5.23-4.85 (6.5H, m), 4.40 (1H, m), 3.30 (2H, m), 2.92-2.65

(2H, m), 2.10-1.98 (1H, m), 1.44 (9H, s), 1.00-0.87 (6H, m).

Following compounds were similarly prepared:

· (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.59 (d, J = 8.7 Hz, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.13 (d, J = 8.7 Hz, 1H), 7.98 (d, J = 8.7 Hz, 1H), 7.88 9d, J = 7.8 Hz, 1H), 7.78 (m, 1H), 7.63 (m, 1H), 7.22-7.15 (m, 4H), 6.96-6.81 (m, 6H), 4.99-4.81 (m, 4H), 4.40 (d, J = 10.1 Hz, 1H), 4.21 (d, J = 10.0 Hz, 1H), 3.44 (d, J = 17.9 Hz, 1H), 3.24 (d, J = 17.9 Hz, 1H), 3.03 (dd, J = 17.0, 4.6 Hz, 1H), 2.76 (dd, J = 17.0, 5.5 Hz, 1H), 2.30(m, 1H), 1.45 (s, 9H), 1.10 (m, 6H)

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.68 (d, J = 8.7 Hz, 1H), 8.32-8.26 (m, 2H), 8.17 (d, J = 8.7 Hz, 1H), 7.91 (m, 2H), 7.80 (m, 1H), 7.66 (m, 1H), 7.28 (m, 4H), 7.02-6.87 (m, 6H), 5.01-4.77 (m, 4H), 4.38-4.30 (m, 2H), 3.50-3.38 (ABq, J = 17.9 Hz, 2H), 3.06-3.02 (m, 1H), 2.84-2.80 (m, 1H), 2.34 (m, 1H), 1.44 (s, 9H), 1.14 (m, 6H)

· (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenoxymethy-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid.

From more polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.01 (d, J = 9.2 Hz, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.55 (d, J = 8.3 Hz, 1H),

8.18-8.07 (m, 3H), 7.87 (m, 1H), 7.73 (m, 1H), 7.28-7.14 (m, 4H), 6.96-6.75 (m, 6H), 5.00-4.75 (m, 4H), 4.42 (d, J = 10.6 Hz, 1H), 4.22 (d, J = 10.6 Hz, 1H), 3.47-3.35 (ABq, J = 17.9 Hz, 2H), 2.82 (dd, J = 17.0, 6.4 Hz, 2.56 (m, 1H), 2.33 (m, 1H), 0.98 (m, 6H).

From less polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.06 (d, J = 9.2 Hz, 1H), 8.88 (d, J = 7.8 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 8.22-8.07 (m, 3H), 7.87 (m, 1H), 7.73 (m, 1H), 7.17 (m, 4H), 6.91-6.78 (m, 6H), 4.98-4.90 (ABq, J = 17.9 Hz, 2H), 4.77 (m, 2H), 4.35 (d, J = 10.6 Hz, 1H), 4.20 (d, J = 10.6 Hz, 1H), 3.47-3.35 (ABq, J = 18.3 Hz, 2H), 2.89 (dd, J = 17.0, 6.4 Hz, 2.61 (dd, J =17.0, 6.4, 1H), 2.31 (m, 1H), 0.98 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H).

· (3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (d, J = 8.3 Hz), 7.94 (d, J = 8.3 Hz), 7.88 (d, J = 7.4 Hz, 1H), 7.74 (d, J = 9.7 Hz, 1H), 7.61-7.44 (m, 4H), 7.35-7.18 (m, 8H), 6.23 (d, J = 8.7 Hz, 1H), 4.95 (m, 1H), 4.76 (m, 1H), 4.49-4.41 (ABq, J = 17.5 Hz, 2H), 3.49-3.41 (m, 2H), 3.22-3.12 (m, 2H), 2.92 (dd, J = 17.0, 4.2 Hz, 1H), 2.52 (dd, J = 17.0, 5.1 Hz, 1H), 2.13 (m, 1H), 1.37 (s, 9H), 1.04 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 8.3 Hz, 1H), 7.83 (m, 2H), 7.57-7.47 (m, 4H), 7.38-7.22 (m, 9H), 6.64 (d, J = 9.2 Hz, 1H), 5.00-4.87 (m, 3H), 4.72 (m, 1H), 3.60 (d, J = 17.9 Hz, 1H), 3.36 (d, J = 14.2 Hz, 1H), 3.20 (d, J = 14.2 Hz, 1H), 3.12 (d, J = 17.9

Hz, 1H), 2.69 (dd, J = 17.0, 4.6 Hz, 1H), 2.28-2.18 (m, 2H), 1.38 (s, 9H), 1.06 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.9 Hz, 3H).

· (3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester (more polar isomer)

^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 1H), 7.62 (d, J = 15.6 Hz, 1H), 7.50 (m, 1H), 7.38-7.21 (m, 12H), 6.39 (d, J = 15.6 Hz, 1H), 5.90 (d, J = 9.2 Hz, 1H), 4.76 (m. 2H), 4.49-4.41 (ABq, J = 17.4 Hz, 2H), 3.42-3.38 (m, 2H), 3.17 (d, J = 14.2 Hz, 1H), 3.09 (d, J = 17.9 Hz, 1H), 2.91 (dd, J = 17.4, 4.6 Hz, 1H), 2.52 (dd, J = 17.4, 5.0 Hz, 1H), 2.04 (m, 1H), 1.41 (s, 9H), 0.90 (m, 6H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.61 (d, 1H), 7.52 (d, 1H), 7.41 (d, 1H), 7.28 (m, 12H), 6.64-6.41 (m, 2H), 5.09-4.99 (ABq, J = 17.4 Hz, 2H), 4.81 (m, 1H), 4.69 (m, 1H), 3.50 (d, J = 17.9 Hz, 1H), 3.34 (d, J = 14.2 Hz, 1H), 3.17 (d, J = 14.2 Hz, 1H), 3.04 (d, J = 17.9 Hz, 1H), 2.74 (dd, J = 17.0, 4.2 Hz, 1H), 2.22 (m, 2H), 1.39 (s, 9H), 0.97-0.88 (m, 6H).

· (3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 9.3 Hz, 1H), 7.38-7.23 (m, 13H), 4.80-4.63 (m, 2H), 4.56-4.46 (ABq, J = 17.1 Hz, 2H), 4.21-4.10 (m, 2H), 3.83 (m, 2H), 3.41-3.37 (m, 1H), 3.19 (d, J = 14.2 Hz, 1H), 2.90-2.83 (m, 2H), 2.53 (m, 1H), 1.76 (m, 1H), 1.41 (s,

9H), 0.83 (d, J = 6.8 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 9.2 Hz, 1H), 7.36-7.26 (m, 13H), 5.05-4.95 (m, 3H), 4.74 (m, 1H), 4.17 (m, 2H), 3.96 (m, 1H), 3.41-2.99 (m, 4H), 2.70 (m, 1H), 2.19 (m, 1H), 1.79 (m, 1H), 1.39 (s, 9H), 0.86 (d, J = 6.4 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H).

· (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.59 (d, J = 8.7 hz, 1H), 8.31 (d, J = 8.7 Hz, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.12 (d, J = 8.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.78-7.72 (m, 2H), 7.62 (m, 1H), 7.33-7.27 (m, 3H), 5.20-5.05 (m, 3H), 4.92-4.89 (m, 2H), 3.47-3.34 (m, 2H), 2.95 (dd, J = 17.0, 4.6 Hz, 1H), 2.73 (dd, J = 17.0, 5.1 Hz, 1H), 2.28 (m, 1H), 1.45 (s, 9H), 1.07 (m, 6H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.58 (d, J = 9.2 Hz, 1H), 8.28-8.24 (m, 2H), 8.12 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.75-7.59 (m, 3H), 7.31-7.25 (m, 3H), 5.12-4.89 (m, 5H), 3.46-3.41 (m, 2H), 2.92 (dd, J = 17.0, 5.1 Hz, 1H), 2.78 (dd, J = 17.0, 5.5 Hz, 1H), 2.30 (m, 1H), 1.44 (s, 9H), 1.10 (m, 6H)

· (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 9.2 Hz,

1H), 8.32 9d, J = 8.3 Hz, 1H), 8.26 (d, J = 8.3 Hz, 1H), 8.12 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.80-7.63 (m, 3H), 7.36-7.18 (m, 8H), 4.82 (m, 1H), 4.72 (m, 1H), 4.47-4.37 (ABq, J = 17.0 Hz, 2H), 3.47 (d, J = 17.9 Hz, 1H), 3.41 (d, J = 13.8 Hz, 1H), 3.19 (d, J = 14.2 Hz, 1H), 3.14 (d, J = 17.9 Hz, 1H), 2.94 (dd, J = 17.4, 4.1 Hz, 1H), 2.53 (dd, J = 17.0, 5.0 Hz, 1H), 2.18 (m, 1H), 1.45 (s, 9H), 0.98 (m, 6H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 9.2 Hz, 1H), 8.28-8.23 (m, 2H), 8.12 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.73 (m, 1H), 7.62-7.55 (m, 2H), 7.31-7.17 (m, 8H), 5.06-4.98 (ABq, J = 17.0 Hz, 2H), 4.84 (m, 1H), 4.69 (m, 1H), 5.54 (d, J = 17.9 Hz, 1H), 3.29 (d, J = 14.2 H, 1H), 3.16 (d, J = 14.2 Hz, 1H), 3.10 (d, J = 17.9 Hz, 1H), 2.70 (dd, J = 17.0, 4.1 Hz, 1H), 2.21 (m, 1H), 2.11 (dd, J = 17.0, 5.1 Hz, 1H), 1.38 (s, 9H), 0.98 (m, 6H).

. (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid t-butyl ester

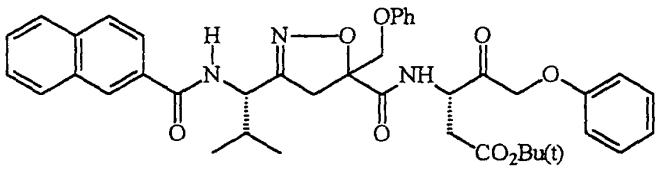
More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.47 (d, J = 9.2 Hz, 1H), 8.32 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.7 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.78 (m, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.29-7.17 (m, 4H), 7.06 (t, J = 7.4 Hz, 1H), 6.96 (t, J = 7.4 Hz, 1H), 6.78 (d, J = 8.3 Hz, 2H), 4.81-4.72 (m, 2H), 4.47-4.28 (ABq, J = 17.9 Hz, 2H), 3.42 (d, J = 17.9 Hz, 1H), 3.34 (d, J = 14.2 Hz, 1H), 3.15 (d, J = 13.7 Hz, 1H), 3.10 (d, J = 17.9 Hz, 1H), 2.94 (dd, J = 17.4, 4.1 Hz, 1H), 2.64 (dd, J = 17.4, 5.5 Hz, 1H), 2.15 (m, 1H), 1.43 (s, 9H), 0.95 (m, 6H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.50 (d, J = 9.2 Hz, 1H), 8.24 (d, J = 8.3 Hz, 1H), 8.18 (d, J = 8.3 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.74 (m, 1H), 7.62-7.56 (m, 2H), 7.29-7.16 (m, 5H), 6.88 (t, J = 7.4 Hz, 1H), 6.78 (d, J = 7.8 Hz, 2H), 4.81-4.66 (m, 4H), 3.46 (d, J = 17.9 Hz, 1H), 3.29 (d, J = 13.8 Hz, 1H), 3.15 (d, J = 13.8 Hz, 1H), 3.07 (d, J = 17.9 Hz, 1H), 2.76 (dd, J = 17.0, 4.1 Hz, 1H), 2.21-2.09 (m, 2H), 1.37 (s, 9H), 0.93 (m, 6H).

. (3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid t-butyl ester

Diastereomeric mixture: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (m, 1H), 7.96-7.50 (m, 7H), 6.85-6.73 (m, 1H), 5.10-4.97 (m, 2H), 4.66 (m, 1H), 3.40 (m, 2H), 2.94-2.60 (m, 2H), 2.32-2.14 (m, 1H), 2.22 & 2.10 (two s, 3H), 1.43 & 1.42 (two s, 9H), 1.10-0.95 (m, 6H).

Example 23: Synthesis of (3S)-3-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonyl-amino)-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-5-phenoxy-pentanoic acid t-butyl ester



The title compound was prepared with convetional EDC coupling of 3-[2-methyl-(1S)-1-(naphthalene-2-car-bonylamino)-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carboxylic acid (1.00g, 2.24 mmol) and (3S)-3-amino- 4-hydroxy-5- phenoxy-pentanoic acid t-butyl ester (630 mg, 1.0 eq), EDC (558 mg, 1.3 eq), HOBt(394 mg, 1.3 eq) and triethylamine

(0.94 mL, 3.0 eq) in DMF (5 mL). Usual workup followed by flash chromatography gave 1.44g of coupled product. The coupled product and Dess-Martin reagent (2.15g, 2.5 mol eq) in dry CH_2Cl_2 (25mL) under N_2 at room temperature was stirred for 1h, then quenched with isopropyl alcohol(3 mL). Usual extractive workup followed by flash chromatography (36% ethyl acetate-hexane) gave 1.27g of the title compound as diastereomeric mixture. Preparative HPLC (36% ethyl acetate-hexanes, 10 mL/min, 278 nm UV detection) afforded less polar (352 mg) and more polar (536 mg) diastereomers.

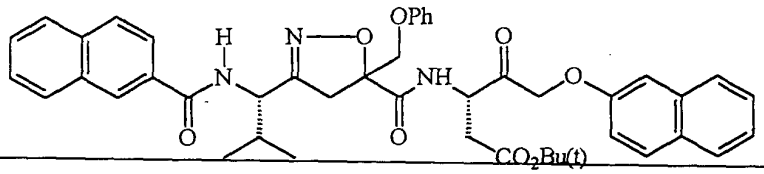
Less polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s), 7.93-7.81 (5H, m), 7.58-7.51 (2H, m), 7.28-7.21 94H, m), 6.99-6.76 (7H, m), 5.00-4.98 (2H, m), 4.79-4.66 (2H, ABq, J = 16.6 Hz), 4.35-4.29 (2H, ABq, J = 10.3 Hz), 3.40 (2H, s), 3.02-2.98 (1H, dd, J = 16.6, 4.9 Hz), 2.84-2.79 (1H, dd, J = 16.6, 4.7 Hz), 2.30 (1H, m), 1.41 (9H, s), 1.12-1.07 (6H, two d, J = 6.8 Hz).

More polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s), 7.99-7.82 (5H, m), 7.59-7.53 (2H, m), 7.26-7.18 (4H, m), 6.97-6.83 (6H, m), 6.68 (1H, d, J = 8.3 Hz), 5.01-4.95 (3H, m), 4.83 (1H, d, J = 17.1 Hz), 4.42 (1H, d, J = 9.8 Hz), 4.23 (1H, d, J = 9.8 Hz), 3.49-3.32 (2H, ABq, J = 18.1 Hz), 3.06-3.02 (1H, dd, J = 17.1, 4.4 Hz), 2.76-2.72 (1H, dd, J = 17.1, 5.4 Hz), 2.24 (1H, m), 1.45 (9H, s), 1.10-1.02 (6H, two d, J = 6.8 Hz).

The following compounds were prepared similarly:

· (3S)-3-{3-[2-methyl-(1S)-1-(naphthalene-2-carbonylamino)-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-5-(2-naphthyloxy)-pen

tanoic acid-t-butyl ester



Less polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.27(1H, s), 7.89 (8H, m), 7.56-7.26 (6H, m), 7.23-6.87 (5H, m), 6.74 (1H, d, J = 9.3 Hz), 5.04-4.95 (2H, m), 4.92-4.80 (2H, ABq, J = 16.6 Hz), 4.37-4.30 (2H, ABq, J = 23.4, 10.3 Hz), 3.43-3.38 (2H, ABq, J = 22.5, 17.8 Hz), 3.05-3.00 (1H, dd, J = 16.6, 4.9 Hz), 2.86-2.82 (1H, dd, J = 16.6, 4.9 Hz), 2.25 (1H, m), 1.42 (9H, s), 1.09-1.05 (6H, two d, J = 6.8, 6.7 Hz).

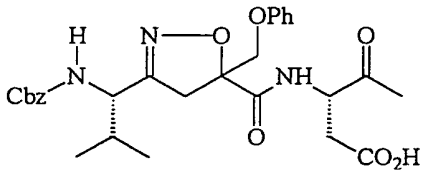
More polar diastereomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, s), 8.02-7.55 (10H, m), 7.41-7.05 (6H, m), 6.89-6.66 (4H, m), 5.10-4.94 (4H, m), 4.41 (1H, d, J = 9.8 Hz), 4.23 91H, d, J = 10.3 Hz), 3.50-3.34 (2H, ABq, J = 17.6 Hz), 3.09-3.05 (1H, dd, J = 17.1, 4.4 Hz), 2.79-2.74 (1H, dd, J = 17.1, 5.4 Hz), 2.25 (1H, m), 1.45 (9H, s), 1.10-1.02 (6H, two d, J = 6.8 Hz).

. (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid t-butyl ester

More polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.60 (d, J = 9.2 Hz, 1H), 8.32-8.25 (m, 2H), 8.13 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.79-7.62 (m, 3H), 7.27 (m, 2H), 6.97 (m, 1H), 6.88 (m, 2H), 5.04-4.72 (m, 5H), 3.48-3.34 (m, 2H), 3.00 (dd, J = 17.0, 4.6 Hz, 1H), 2.77 (dd, J = 17.0, 5.5 Hz, 1H), 2.27 (m, 1H), 1.45 (s, 9H), 1.06 (m, 6H).

Less polar isomer: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.58 (d, J = 9.2 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.78-7.59 (m, 3H), 7.22 (m, 2H), 6.92 (m, 1H), 6.82 (m, 2H), 5.04-4.88 (m, 3H), 4.82-4.69 (ABq, J = 17.0 Hz, 2H), 3.45-3.33 (m,2H), 2.99 (dd, J = 16.5, 4.6 Hz, 1H), 2.78 (dd, J = 16.5, 5.1 Hz, 1H), 2.26 (m, 1H), 1.42 (s, 9H), 1.06 (m, 6H)

Example 24: Synthesis of (3S)-3-{3-[(1S)-1-benzyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid



A solution of (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid t-butyl ester (less polar diastereomer) (44mg) in CH_2Cl_2 (2 mL) at 0°C was treated with TFA (1 mL). The reaction was stirred for 2h while slowly warming to room temperature. Concentration gave the title compound (compound 2, quantitative)

^{1}H NMR (500 MHz, CD_3OD) δ 7.35-6.90 (10H, m), 5.11 (2H, s), 4.53 (1H, m), 4.47 (1H, m), 4.23 (2H, dd), 2.86 (1H, dd), 2.54 (1H, dd), 2.24 (3H, s), 2.00 (1H, m), 1.00 and 0.97 (6H, two d); MS $[M+Na]^+$ 562

The following compound was prepared similarly from more polar isomer:

. (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid (compound 3).

^{1}H NMR (500 MHz,) δ 8.76 (1H,d, J = 7.8 Hz), 7.76 (1H, d, J = 8.8 Hz), 7.36-6.87 (10H, m), 5.06 (2H, m), 4.50 (1H, m), 4.32 (1H, m), 4.16 (2H, m), 3.21 (2H, app s), 2.79 (1H, m), 2.06 (3H, s), 1.89 (1H, m), 0.91 (3H, d, J = 6.3 Hz), 0.80 (3H, d, J = 6.3 Hz).

Following final compounds were obtained by similar TFA deprotection of corresponding t-butyl ester.

. (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid (compound 1, diastereomeric mixture)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8. 49 (1H, m), 7.72 (1H, m), 7.35 (5H, m), 5.03 (3H, m), 4.40 (1H, m), 4.15 (1H, m), 3.24 (2H, m), 2.54 (2H, m), 2.04 and 1.95 (3H, wo s), 1.88 (1H, m), 0.90-0.81 (6H, m): MS $[M+Na]^+$ 456

. (3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlrobenzoyloxy)-pentanoic acid (compound 6, diastereomeric mixture)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (1H, br s), 7.75 (1H, m), 7.61-7.30 (8H, m), 5.30-5.00 (5H, m), 4.70 (1H, m), 4.16 (1H, m), 2.66 (2H, m), 1.90 (1H, m), 0.95-0.79 (6H, m): MS $[M+Na]^+$ 644

. (3S)-3-{3-[(1S)-1-(naphthalene-1-carbonylamino)-2-methyl-propyl]-5-penoxy methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 7, diastereomeric mixture)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92-8.55 (2H, m), 8.15-7.98 (3H, m), 7.63-7.55 (4H, m), 7.25-7.15 (4H, m), 6.95-6.74 (6H, m), 5.20-4.15 (6H, m), 2.80-2.55 (2H, m), 2.05 (1H, m), 1.05-0.89 (6H, m): MS $[M+Na]^+$ 674.

. (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 8)

From less polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.93 (1H, d, J = 7.8 Hz), 8.79 (1H, d, J = 8.3 Hz), 8.48 (1H, s), 8.05-7.94 (4H, m), 7.64-7.58 (2H, m), 7.30-7.17 (4H, m), 6.94-6.83 (6H, m), 4.96 (2H, app s), 4.78 (1H, m), 4.73 (1H, m), 4.36 (1H, d, J = 10.2 Hz), 4.22 (1H, d, J = 10.2 Hz), 3.37 (2H, app s), 2.91 (1H, dd, J = 16.6, 6.4 Hz), 2.62 (1H, dd, J = 16.6, 5.9 Hz), 2.12 (1H, m), 1.00 (3H, d, J = 6.3 Hz), 0.87 93H, d, J = 6.3 Hz): MS $[M+Na]^+$ 674

From more polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (1H, d, J = 8.3 Hz), 8.79 (1H, d, J = 8.8 Hz), 8.43 (1H, s), 8.00-7.80 (4H, m), 7.61 (2H, m), 7.23-7.17 94H, m), 6.93-6.77 (6H, m), 4.99 (1H, d, J = 17.6 Hz), 4.86 (1H, d, J = 18.1 Hz), 4.79 (1H, m), 4.72 (1H, m0, 4.43 (1H, d, J = 10.7 Hz), 4.20 (1H, d, J = 10.2 Hz), 2.81 (1H, dd), 2.56 (1H, dd), 2.17 (1H, m), 1.01 (3H, d, J = 6.3 Hz), 0.99 (3H, d, J = 6.3 Hz): MS $[M+Na]^+$ 674

. (3S)-3-{3-[(1S)-1-(naphthalene-1-carbonylamino)-2-methyl-propyl]-5-penoxy methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlrobenzoyloxy)-pentanoic acid (compound 9)

From less polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.08 (1H, d, J = 7.8 Hz), 8.87 (1H, d, J = 8.8 Hz), 8.55 (1H, s), 8.10-8.01 (4H, m), 7.68 7.58 (5H, m), 7.26 (2H, t, J = 7.8 Hz), 6.98-6.92 (3H, m), 5.27 (2H, ABq, J = 16.6 Hz0, 4.82-4.78 (2H, m), 4.43 (1H, d, J = 10.7 Hz), 4.29 (1H, d, J = 10. 3 Hz), 3.44 (2H, ABq, J = 18.1 Hz), 3.01 (1H, dd, J = 17.1, 6.4 Hz), 2.67 (1H, dd, J = 17.1, 6.3 Hz), 2.21 (1H, m), 1.07 (3H, d, J = 6.2 Hz), 0.97 (3H, d, J = 6.2 Hz): MS $[M+Na]^+$ 770

From more polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.97 (1H, d, J = 7.8 Hz), 8.85 (1H, d, J = 8.3 hz), 8.50 (1H, s), 8.09-7.96 (4H. m), 7.67-7.60 (5H, m), 7.32 (2H, t, J = 6.3 Hz), 7.00 (3H, m), 5.38 (1H, d, J = 17.1 Hz), 5.13 (1H, d, J = 17.1 Hz), 4.92 (1H, d, J = 6.3 Hz), 4.79 (1H, t, J = 7.8 Hz), 4.55 (1H, d, J = 9.7 Hz), 4.28 (1H, d, J = 8.7 Hz), 3.48 (1H, d, J = 18.1 Hz), 3.38 (1H, d, J = 18.1 Hz), 2.87 (1H, dd, J = 17.1, 4.9 Hz), 2.60 (1H, dd, J = 17.1, 4.9 Hz), 2.25 (1H, m), 1.07 (6H, m): MS $[M+Na]^+$ 770

. (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 10, diastereomeric)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72-8.55 (2H, m), 8.38 (1H, s), 8.04-7.85 (4H, m), 7.62 (2H, m), 7.25-7.12 (7H, m), 6.91-6.70 (3H, m), 4.79-4.51 (4H, m), 3.40-3.05 (4H, m), 2.73-2.23 (2H, m), 2.01 (1H, m), 0.94-0.70 (6H, m): MS $[M+Na]^+$ 658

. (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoylo xy)-pentanoic acid (compound 11)

From less polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (1H, d, J = 8.8 Hz), 8.59 (1H, d, J = 8.3 Hz), 8.40 (1H, s), 8.05-7.87 (4H, m), 7.63-7.54 (5H, m), 7.21-7.13 (5H, m), 5.98 (2H, ABq, J = 17.1 Hz), 4.74 (1H, m), 4.64 (1H, m), 3.25-3.10 (4H, m), 2.62 (1H, dd, J = 17.1, 6.3 Hz), 2.37 (1H, dd, J = 16.6, 5.4 Hz), 2.06 (1H, m), 0.93 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 6.2 Hz): MS $[M+Na]^+$ 754

From more polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (1H, d, J = 8.3 Hz), 8.59 91H, d, J = 8.8 Hz), 8.41 (1H, s), 8.01-7.87 (4H, m), 7.62-7.53 (5H, m), 7.29-7.21 (5H, m), 4.70-4.55 (4H, m), 3.44-3.10 (4H, m), 2.72-2.67 (1H, dd, J = 16.6, 7.3 Hz), 2.38-2.34 (1H, dd, J = 16.6, 7.3 Hz), 2.05 (1H, m), 0.97 (3H, d, J = 6.3 Hz), 0.79 (3H, d, J = 6.3 Hz); MS $[M+Na]^+$ 754.

. (3S)-3-{3-[(1S)-1-(quinoline-2-yl-carbonylamino)-2-methyl-propyl]-5-phenox ymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid (compound 13, diastereomeric mixture)

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (1H, m), 8.82 91H, br), 8.57 (1H, m), 8.16-7.74 95H, m), 7.26-7.12 (4H, m), 6.89-6.69 (6H, m), 5.10-4.70 (4H, m), 4.48-4.20 (2H, m), 2.87-2.53 (2H, m), 2.32 (1H, m), 0.98-0.85 (6H, m): MS $[M+Na]^+$ 675, $[M+H]^+$ 653.

. (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenox

ymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid
(compound 15)

From less polar t-butyl ester: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.96 (1H, d, J = 7.8 Hz), 8.77 (1H, d, J = 8.3 Hz), 8.47 (1H, s), 8.03-7.57 (9H, m), 7.44 (1H, t, J = 6.8 Hz), 7.34 (1H, t, J = 7.8 Hz), 7.17-7.13 (4H, m), 6.88-6.82 (3H, m), 5.09 (2H, ABq), 4.84 (1H, m), 4.72 (1H, m), 4.38 (1H, d, J = 10.2 Hz), 4.23 (1H, d, J = 10.7 Hz), 2.94 (1H, dd, J = 17.1, 6.8 Hz), 2.65 (1H, dd, J = 16.6, 5.9 Hz), 2.12 (1H, m), 0.97 (3H, d, J = 6.3 Hz), 0.85 (3H, d, J = 6.3 Hz): MS $[M+Na]^+$ 724

From more polar t-butyl ester: ^{1}H NMR (50°C, 300 MHz, DMSO-d_6) δ 8.72 (1H, d), 8.63 (1H, d), 8.41 (1H, s), 7.94-6.72 (19H, m), 5.03 (2H, ABq), 4.88 (1H, m), 4.74 (1H, m), 4.42 91H, d), 4.19 (1H, m), 3.38 (2H, ABq), 2.88 (1H, dd), 2.65 (1H, dd), 2.19 (1H, m), 1.02 (6H, two d):MS $[M+Na]^+$ 724

^{13}C NMR (50°C, 300 MHz, DMSO-d_6) δ 202.1, 171.6, 170.7, 166.6, 159.3, 158.0, 155.6, 134.1, 133.9, 132.0, 131.6, 129.3, 129.1, 128.7, 127.7, 127.5, 127.3, 126.5, 126.2, 124.2, 123.6, 121.1, 118.1, 114.5, 107.4, 87.5, 70.2, 52.9, 34.4, 29.6, 19.4, 18.9.

More polar diastereomer's methyl ester: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s), 8.02-6.68 (20H, m), 5.09-4.95 (2H, ABq, J = 16.6 Hz), 5.10 (1H, m), 5.01(1H, m), 4.34 (2H, ABq, J = 10.3 Hz), 3.70 (3H, s), 3.50-3.33 (2H, ABq, J = 17.6 Hz), 3.13 (1H, dd, J = 17.1, 4.9 Hz), 2.90 (1H, dd, J = 17.1, 5.9 Hz), 2.23 (1H, m), 1.08 and 1.02 (6H, two d, J = 6.8 Hz).

· (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-naphthyloxy)-pentanoic acid (compound 16, diasteromeric mixture)

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^{1}H NMR (500 MHz, DMSO-d_6) δ 9.02-8.18(3H, m), 8.05-6.80 (18H, m), 5.15-4.15 (6H, m), 2.90-2.55 (2H, m), 2.14 (1H, m), 1.05-0.82 (6H, m).

· (3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid (compound 19, diasteromeric mixture)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95-8.46 (3H, m), 8.09-7.07 (13H, m), 5.21-4.75 (5H, m), 2.95-2.64 (2H, m), 2.19 (1H, m): MS $[M+H]^+$ 596

· (3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 20, diasteromeric mixture)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.76-8.69 (m, 2H), 8.45 (m, 1H), 8.04-7.90 (m, 5H), 7.61 (m, 2H), 7.31-7.19 (m, 2H), 6.97-6.81 (m, 3H), 5.09-4.68 (m, 5H), ~3.3 (m, 2H), 2.82 (m, 1H), 2.64 (m, 1H), 2.15 ((m, 1H), 1.00-0.84 (m, 6H): MS [M+Na] = 568

· (3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 22, diastereomeric mixture)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.48 (br s, 1H), 8.00 (m, 1H), 7.61-7.54 (m, 3H), 7.30-7.15 (m, 11H), 4.93-4.32 (m, 4H), 3.34-2.90 (m, 4H), 2.78 (m, 1H), 1.78 (m, 1H), 0.90-0.60 (m, 6H): MS [M+Na] = 732

. (3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid(compound 23)

From more polar t-butyl ester: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.80 (d, J = 8.3 Hz, 1H), 8.63 (d, J = 7.8 Hz, 1H), 8.02 (m, 3H), 7.64-7.20 (m, 12H), 4.81-4.55 (m, 4H), 3.39 (m, 2H), 3.12 (m, 2H), 2.73 (m, 1H), 2.43 (m, 1H), 1.98 (m, 1H), 0.99 (d, J = 4.6 Hz, 3H), 0.79 (d, J = 4.5 Hz, 3H): MS [M+Na] = 754

From less polar t-butyl ester: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.77 (d, J = 8.7 Hz, 1H), 8.62 (d, J = 8.3 Hz, 1H), 8.08-7.97 (m, 4H), 7.61-7.21 (m, 12H), 5.00 (m, 2H), 4.77-4.67 (m, 2H), 3.39-3.27 (m, 2H), 3.15-3.11 (m, 2H), 2.64 (m, 1H), 2.40 (m, 1H), 1.99 (m, 1H), 0.96 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.9 Hz, 3H).

. (3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 28)

Fom more polar t-butyl ester: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.55 (d, J = 8.7 Hz, 1H), 8.28 (d, J = 8.7 Hz, 1H), 7.60-7.19 (m, 14H), 6.70 (d, J = 15.6 Hz, 1H), 4.71-4.49 (m, 4H), ~3.3 (m, 2H), 3.08 (m, 2H), 2.71 (m, 1H), 2.40 (m, 1H), 1.90 (m, 1H), 0.86 (d, J = 6.4 Hz, 3H), 0.74 (d, J = 6.4 Hz, 3H): MS [M+H] = 708

From less polar t-butyl ester: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.53 (d, J = 8.3 Hz, 1H), 8.28 (d, J = 8.7 Hz, 1H), 7.61-7.16 (m, 14H), 6.69 (d, J = 16.9 Hz, 1H), 4.99-4.92 (ABq, J = 17.4 Hz, 2H), 4.72 (m, 1H), 4.53 (m, 1H), 3.36 (d, J = 17.9 Hz, 1H), 3.23 (d, J = 13.8 Hz, 1H), 3.10-3.04 (m, 2H), 2.61 (dd, J = 17.0, 6.4 Hz, 1H), 2.37 (dd, J = 17.0, 6.0 Hz, 1H), 1.90 (m, 1H), 0.79 (m, 6H).

· (3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 29, diastereomeric)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.75 and 7.69 (m, 1H), 7.61-7.13 (m, 13H), 5.00 and 4.70 (m, 1H), 4.64 (m, 2H), 4.22-3.78 (m, 4H), 1.79 (m, 1H), 0.90 (m, 6H):MS [M+H] = 732.

· (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 30)

From more polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.94 (d, J = 9.7 Hz, 1H), 8.75 (d, J = 7.8 Hz, 1H), 8.55 (d, J = 8.8 Hz, 1H), 8.18-8.05 (m, 3H), 7.85 (m, 1H), 7.55 (m, 3H), 5.22-5.06 (m, 3H), 4.83-4.70 (m, 2H), 3.35 (m, 2H), 2.80 (m, 1H), 2.61 (m, 1H), 2.31 (m, 1H), 0.95 (m, 6H):MS [M+H] = 643

From less polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.07 (d, J = 9.2 Hz, 1H), 8.76 (d, J = 8.3 Hz, 1H), 8.56 (d, J = 8.7 Hz, 1H), 8.20-8.07 (m, 3H), 7.87 (m, 1H), 7.72 (m, 1H), 7.62-7.54 (m, 3H),

5.21-5.06 (m, 3H), 4.84-4.70 (m, 2H), 3.44-3.27 (m, 2H), 2.85 (dd, J = 17.0, 6.0, 1H), 2.66 (dd, J = 17.0, 6.9 Hz, 1H), 2.29 (m, 1H), 0.98 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.4 Hz, 6H)

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 31)

From more polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.95 (d, 1H), 8.72 (d, 1H), 8.55 (d, 1H), 8.20-8.05 (m, 3H), 7.86 (m, 1H), 7.72 (m, 1H), 7.24-6.74 (m, 5H), 5.11-4.70 (m, 5H), 3.34 (m, 2H), 2.80 (m, 1H), 2.62 (m, 1H), 2.30 (m, 1H), 0.95 (m, 6H):MS [M+H] = 547

From less polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.03 (d, 1H), 8.74 (d, 1H), 8.56 (d, 1H), 8.20-8.07 (m, 3H), 7.87 (m, 1H), 7.73 (m, 1H), 7.23 (m, 2H), 6.88 (m, 3H), 5.09-4.71 (m, 5H), 3.34 (m, 2H), 2.85 (m, 1H), 2.65 (m, 1H), 2.27 (m, 1H), 0.96-0.87 (m, 6H).

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid (compound 32)

From more polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.91 (d, J = 9.2 Hz, 1H), 8.59-8.52 (m, 2H), 8.17-8.06 (m, 3H), 7.87 (m, 1H), 7.72 (m, 1H), 7.58-7.53 (m, 5H), 4.69-4.51 (m, 4H), 3.40 (m, 2H), 3.16 (m, 1H), 2.69 (m, 1H), 2.37 (m, 1H), 2.19 (m, 1H), 0.91-0.80 (m, 6H): MS [M+H] = 733

From less polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.92 (d, J = 9.2 Hz, 1H), 8.56(m, 2H), 8.19-8.07 (m, 3H), 7.87 (m, 1H), 7.73 (m, 1H),

7.60-7.54 (m, 3H), 7.22-7.07 (m, 5H), 5.01-4.93 (ABq, J = 16.5 Hz, 2H), 4.75-4.62 (m, 2H), 3.46 (d, J = 18.4 Hz, 1H), 3.23-3.07 (m, 3H), 2.62 (dd, J = 17.0, 6.9 Hz, 1H), 2.37 (dd, J = 17.0, 6.0 Hz, 1H), 2.21 (m, 1H), 0.86-0.83 (m, 6H).

. (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 33)

From more polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.91 (d, J = 9.2 Hz, 1H), 8.62 (d, J = 8.3 Hz, 1H), 8.52 (d, J = 8.7 Hz, 1H), 8.15 (d, J = 8.3 Hz, 1H), 8.07 (m, 2H), 7.86 (m, 1H), 7.72 (m, 1H), 7.26-7.09 (m, 7H), 6.86 (m, 1H), 6.69 (d, J = 8.3 Hz, 2H), 4.71-4.63 (m, 2H), 4.54-4.46 (ABq, J = 17.9 Hz, 2H), 3.42 (d, J = 17.9 Hz, 1H), 3.29 (d, J = 13.8 Hz, 1H), 3.15 (d, J = 18.4 Hz, 1H), 3.09 (d, J = 14.3 Hz, 1H), 2.72 (dd, J = 17.0, 6.9 Hz, 1H), 2.36 (dd, J = 17.0, 6.0 Hz, 1H), 2.15 (m, 1H), 0.88 (d, J = 6.9 Hz, 3H), 0.75 (d, J = 6.9 Hz, 3H): MS [M+H] = 637

From less polar isomer: ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.88 (d, J = 9.6 Hz, 1H), 8.54 (m, 2H), 8.18-8.06 (m, 3H), 7.87 (m, 1H), 7.72 (m, 1H), 7.28-6.78 (m, 10H), 4.78-4.63 (m, 4H), 3.45 (d, J = 18.3 Hz, 1H), 3.26-3.06 (m, 3H), 2.66-2.62 (dd, J = 17.0, 6.9 Hz, 1H), 2.44-2.39 (dd, J = 17.0, 5.5 Hz, 1H), 2.17 (m, 1H), 0.80 (m, 6H).

. (3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-(1-imid azolylmethyl)-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-penta noic acid (compound 34, diastereomeric mixture)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.09-6.60 (m, 16H), 4.92-4.62 (m, 6H), 3.50 (m, 2H), 2.85-2.20 (m, 3H), 0.93 (m, 6H): MS [M+H] = 627

· (3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid (compound 35, diastereomeric mixture)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.77 (m.1H), 8.45 (m, 2H), 8.07-7.89 (m, 4H), 7.61 (m, 2H), 5.06 (m, 1H), 4.72 (m, 1H), 4.46 & 4.38 (two m, 1H), ~3.3 (m, isoxazoline CH_2), 2.62 (m, 1H), ~2.49 (m, 1H), 2.13 (m, 1H), 2.09 & 2.05 (two s, 3H), 1.01-0.84 (m, 6H).

· (3S)-3-{3-[(1S)-1-(succinoylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 36, diastereomeric mixture)

^{1}H-NMR (500 MHz,DMSO-d_6) δ 8.56-8.52 (m, 1H), 8.15 (m, 1H), 7.27 (m, 2H), 6.97-6.82 (m, 3H), 4.96-4.83 (m, 2H), 4.77 (m, 1H), 4.58 (m, 1H), 3.58-2.22 (m, 10H), 2.0-1.74 (m, 2H), 1.47 & 1.45 (two s, 3H):MS [M+Na] = 558.

· (3S)-3-{3-[2-methyl-(1S)-1-(succinoylamino)-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (compound 37, diastereomeric mixture)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.62-8.52 (m, 1H), 8.06 (m, 1H), 7.27 (m, 2H), 6.96-6.81 (m, 3H), 4.94-4.72 (m, 3H), 4.43-4.32 (m, 1H), 3.38-3.22 (m, 1H), 2.94-2.78 (m, 2H), 2.70-2.22 (m, 5H), 1.95-1.77 (m, 1H), 1.48 & 1.46 (two s, 3H), 0.86-0.70 (m, 6H): MS [M+Na] = 528.

· (3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenylcarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-piperidinyl)-pentanoic acid (compound 38, diastereomeric)

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^{1}H-NMR (500 MHz,DMSO-d_6) δ 8.75 (m, 1H), 8.47 and 8.29 (m, 1H), 8.03-7.23 (m, 12H), 4.65 (m, 2H), 3.11-2.99 (m, 2H), 2.26-2.18 (m, 4H), 1.97 (m, 1H), 1.64-0.79 (m, 12H):MS [M+H] = 627.

Industrial Applicability

Experiment 1: Screening on caspases enzyme inhibiting activity

In the present experiment, recombinant caspases were purified from a transformed bacterium after human caspase genes were cloned into an expression vector pET, and then used in the experiment (Thornberry, N.A. et .al. *Nature*, **1992**, *356*, 768. Thornberry, N.A. *Methods in Enzymology*, **1994**, *615*.).

Enzymatic activity was measured by a known procedure (Walker N.P.C. et al., *Cell* **1994**, *78*, 343). Briefly, 10 ng of recombinant protein was mixed with 50 mM Tris[pH 7.0], 1mM DTT, 0.5 mM EDTA, 10% Glycerol buffer containing 1~100 uM of enzyme substrate, Ac-YVAD-AMC or Ac-DEVD-AMC and then the changes by isolated AMC at 37°C were recorded. The inhibitory activity for caspases was calculated from the early enzyme reaction rate by measuring the changes with fluorescence excited at 380 nM and emitted at 460 nm (Range; Ki<100nM).

Experiment 2) Screening for intracellular inhibitory efficacy for caspases

Inhibitory activity for Caspase-1 was determined by screening the effects of compounds on the IL-1β production in the periphery lymphocytes stimulated with LPS. Briefly, 500,000 cells/ml of human peripheral lymphocytes was treated with various concentration of test compounds for 2 hours and then with 10 ng/ml of LPS. After incubating the cells for 12 hours, the supernatant samples from the media were analysed by immunoantibody analysis (Amersham) in which 100 ng/well of human IL-1β antibody is coated (Range: CIC_{50} : 0.1 ~ 10 μM).

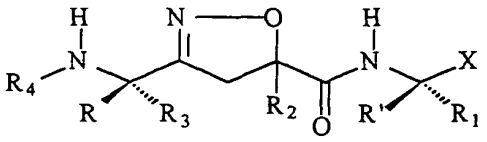
Meanwhile, the efficacy of the compounds on apoptosis was quantified by MTT assay in which cell death and survival ratio depending on the concentration of compounds were analyzed in Jurkat T cell treated with Anti-FAS antibody CH11 which induces cell death (Effective range; 1.0 ~ 10 μM).

Table 1

compound	Ki for caspase-1	CIC_{50} (IL-1β production)	ED_{50} FAS Induced cell death
11	< 100 *n*M	0.1-10 μM	1-10 μM
13	"	"	"
18	"	"	"
21	"	"	"
22	"	"	"
23	"	"	"
28	"	"	"
30	"	"	"
32	"	"	"
33	"	"	"
36	"	"	"
37	"	"	"

Claims

1. An isoxazoline derivative of the formula (I), the pharmaceutically acceptable salts, the esters and the stereochemically isomeric forms thereof



(I)

in which,

R and R' each independently represents simple alkyl chain (-SAC), simple cycloalkyl (-SCAC), aromatic (-Ar), or simple alkyl chain substituted with aromatic (-SAC-Ar) or hydrogen;

R_1 represents -SAC, -SCAC, -Ar, or -SAC-Ar and/or contains side chain residues of natural amino acids, or represent $-CH_2COOH$;

R_3 represents -SAC, -SCAC, -Ar, or -SAC-Ar and/or contains side chain residues of natural amino acids, or represent $-CH(CH_3)_2$, $-CH_2COOH$, $-(CH_2)_2CO_2H$, $-CH_2C(=O)NH_2$ or $-(CH_2)_2C(=O)NH_2$;

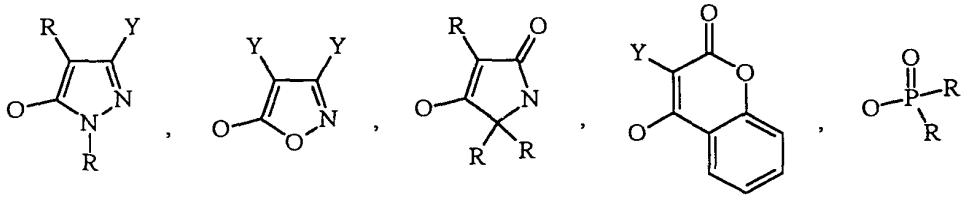
R_2 represents -H, -SAC, -SCAC, -Ar, or -SAC-Ar and contains side chain residues of natural amino acids or $-(CH_2)_n(O)_mR_5$ (in which R_5 = -SAC, -SCAC, -Ar, -SAC-Ar; and n=0, 1, 2; m=0, 1), or $-(CH_2)_nOC(=O)R_6$ (in which R_6 = -SAC, -SCAC, -Ar, or -SAC-Ar; and n=1, 2) or represents $(CH_2)_n(O)_mAr'$ (in which n=0, 1, 2; m=0,1; Ar'=substituted phenyl or imidazole), methyl or hydrogen;

R_4 represents an organic acid acyl group of all the natural amino acids or represents $-C(=O)R_7$ (in which R_7 = -SAC, -SCAC, -Ar, or -SAC-Ar),

$-C(=O)OR_8$ (in which R_8 = -SAC, -SCAC, -Ar, or -SAC-Ar), $-C(=O)NR_9R_{10}$ (in which R_9, R_{10} = -H, -SAC, -SCAC, -Ar, or -SAC-Ar), $-SOR_{11}$ (in which R_{11} = -SAC, -SCAC, -Ar, or -SAC-Ar), or $-SO_2R_{12}$ (in which R_{12} = -SAC, -SCAC, -Ar, or -SAC-Ar);

In cases where R_1 and the adjacent R', and/or R_3 and the adjacent R are connected to each other to form a cyclic compound, R_1-R' or R_3-R together represents $(CH_2)_n$, $(CH_2)_n$-O-$(CH_2)_m$, or $(CH_2)_n$-NR_{13}-$(CH_2)_m$ [in which n+m <9, R_{13} = -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, or -C(=O)-SAC-Ar];

X represents -CN, -CHO, $-C(=O)R_{14}$ [in which R_{14} = -SAC, -SCAC, -Ar, or -SAC-Ar], $-C(=O)OR_{15}$ [in which R_{15} = -SAC, -SCAC, -Ar, or -SAC-Ar], $-CONR_{16}R_{17}$ [in which R_{16} and R_{17} each represents -H, -SAC, -SCAC, -Ar, or -SAC-Ar], $-C(=O)CH_2OR_{18}$ [in which R_{18} represents -SAC, -SCAC, -Ar, or -SAC-Ar], or $-C(=O)CH_2OC(=O)R_{19}$ [in which R_{19} = -SAC, -SCAC, -Ar, or -SAC-Ar]. The invention further encompasses a case where if X represents $-COCH_2$-W, W represents $-N_2$, -F, -Cl, -Br, -I, $-NR_{20}R_{21}$ or $-SR_{22}$ [in which wherein R_{20}, R_{21} and R_{22} each independently represents -SAC, -SCAC, -Ar, or -SAC-Ar or a case where R_{20} and R_{21} are connected to form a cyclic compounds]; W also represents



in which Y represents -OH, OR_{23} (in which R_{23} = -SAC, or -SCAC), $-C(=O)R_{24}$ (in which R_{24} = -H, -SAC, or -SCAC), -F, -Cl, -Br, -I, -CN, -NC, $-N_3$, $-CO_2H$, CF_3, $-CO_2R_{25}$ (in which R_{25} = -SAC, or -SCAC),

-C(=O)NHR$_{26}$ (in which R$_{26}$ = -SAC, or -SCAC), and -C(=O)NR$_{27}$R$_{28}$ (in which R$_{27}$, R$_{28}$ = -SAC, or -SCAC) and can be mono- or poly-substituted at its maximum regardless of the order and the kinds.

2. The compound of formula (I) according to claim 1, in which

a) R and R' represent hydrogen,

b) R$_1$ represents -CH$_2$COOH,

c) R$_2$ represents (CH$_2$)$_n$(O)$_m$Ar' [in which n=1, 2; m=0, 1; Ar'=substituted phenyl or imidazole], methyl or hydrogen,

d) R$_3$ represents -CH(CH$_3$)$_2$, -CH$_2$COOH, -(CH$_2$)$_2$CO$_2$H, -CH$_2$C(O)NH$_2$ or -(CH$_2$)$_2$C(O)NH$_2$,

e) R$_4$ represents -C(=O)(O)$_n$R$_{29}$ [in which n=0,1; R$_{29}$=-Ar, or -SAC-Ar], -SO$_2$R$_{30}$ [in which R$_{30}$ = -Ar, or -SAC-Ar], or -C(=O)NHR$_{31}$, [in which R$_{31}$ = -Ar, or -SAC-Ar],

f) X represents -C(=O)CHN$_2$, -C(=O)CH$_2$Br, -C(=O)CH$_2$Cl, -C(=O)CH$_2$OAr" [Ar" = phenyl] or -C(=O)CH$_2$OC(=O)Ar'" [in which Ar'"=2,6-dichlorophenyl or 2,6-dimethylphenyl].

3. The derivative according to Claim 1, wherein the compound is selected from the group consisting of

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-4-keto-pentanoic acid;

(2S)-2-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonyl-amino}-succinic acid 1-(N-methyl-N-methoxy)-amide;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-phenylmethyloxycarbonylamino-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-1-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-

methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(quinoline-2-yl-carbonylamino)-2-methyl-propyl]-5-phenoxymethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-sulfonylamino)-2-methyl-propyl]-5-penoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-5-phenoxy-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-naphthyloxy)-pentanoic acid;

(3S)-3-{3-[(1S)-1-(2S)-2-acetylamino-succinoylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[(1S)-1-(naphthalene-2-carbonylamino)-2-methyl-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2-naphthyloxy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid (diasteromeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-

methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethyloxycarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(phenylethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoylo xy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphthalenecarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoylo xy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid (diastereomeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid (diastereomeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenesulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid (diastereomeric mixture);

(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)ethylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-((3-indolyl)methylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyl-oxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(cinnamoylamino)-propyl]-5-phenylmethyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;
(3S)-3-{3-[2-methyl-(1S)-1-(phenylmethylsulfonylamino)-propyl]-5-phenyl-

methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

~~(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-~~isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-diazo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-bromo-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(2,6-dichlorobenzoyloxy)-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(quinoline-2-yl-carbonylamino)-propyl]-5-(1-imidazolyl-methyl)-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(2-naphthalenecarbonylamino)-propyl]-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-pentanoic acid;

(3S)-3-{3-[(1S)-1-(succinoylamino)-3-carboxy-propyl]-5-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid;

(3S)-3-{3-[2-methyl-(1S)-1-(succinoylamino)-propyl]-5-methyl-4,5-dihydro-

isoxazole-5-carbonylamino}-4-keto-5-phenoxy-pentanoic acid; and (3S)-3-{3-[2-methyl-(1S)-1-(1-naphtalenylcarbonylamino)-propyl]-5-phenyl-methyl-4,5-dihydro-isoxazole-5-carbonylamino}-4-keto-5-(1-piperidinyl)-pentanoic acid.

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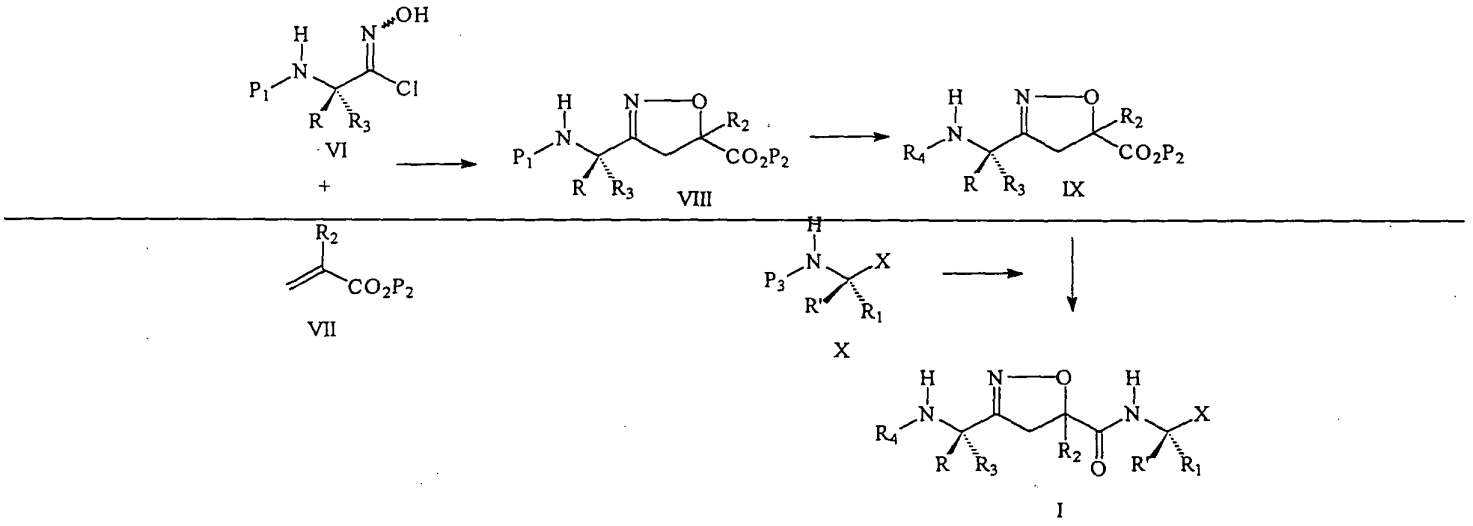
4. A pharmaceutical composition for inhibiting caspases activity which comprises as the active ingredient a therapeutically effective amount of a derivative of formula (1) as claimed in any of Claim 1 to 3 and pharmaceutically acceptable carrier.

5. The composition according to Claim 4, in the form for administration orally, percutaneously, or by parenteral injection.

6. A method of treating patients suffering from the diseases caused by caspases activation, said method comprising the local or systemic administration of a pharmaceutically effective amount of the compound of formula (I) according to any of Claim 1 to 3 or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutical carrier.

7. A process for preparing a derivative of the formula (I), which is characterized in that hydroxamoyl chloride (VI) is reacted with acrylate derivative (VII) to give isoxazoline derivative (VIII), and isoxazoline derivative (VIII) is then deprotected and R_4 is introduced thereinto to give a compound of formula (IX) which is then reacted with a compound of formula (X) and, if necessary, the isoxazoline derivative (VIII) is directly reacted with the compound (X) to give a compound of formula (I), and if necessary, the compound of formula (I) having the protecting group P_1 is converted into other compound having substituent R_4.



in which the sustituents are the same as defined in Claim 1.

Abstract

The present invention relates to an isoxazoline derivative of formula (I), the pharmaceutically acceptable salts, esters and stereochemically isomeric forms thereof, and the use of the derivative in inhibiting the activity of caspases. The derivative according to the present invention can be effectively used in treating diseases due to caspases, such as, for example the disease in which cells are abnormally died, dementia, cerebral stroke, AIDS, diabetes, gastric ulcer, hepatic injure by hepatitis, sepsis, organ transplantation rejection reaction and anti-inflammation.